Novel plant growth regulator effects on yield, senescence and cytokinin homeostasis in wheat, barley and rapid cycling *Brassica rapa*

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Abstract

Two novel plant growth regulators (PGRs), INCYDE and TDZ-K, were tested to verify the claims made for each of them. INCYDE is reported to inhibit cytokinin oxidase/dehydrogenase (CKX) and to enhance yield and growth in *Arabidopsis thaliana* and *Brassica napus* L., and to increase plant resistance to abiotic stress. TDZ-K is reported to delay senescence in wheat leaves by blocking the degradation of photosynthetic complexes within photosystem II. The effect and efficacy of INCYDE and TDZ-K was determined by application to rapid cycling *Brassica rapa* (RCBr) in controlled growth rooms, and by spray application to wheat and barley cultivars in both field and pot trials. Following harvest, the effect of each PGR on growth and yield components was determined. Leaf samples were used to analyse the content of chlorophyll. Wheat and barley grain samples were freeze dried and analysed by LC-MS/MS to determine the content of endogenous cytokinins. Wheat grains and leaves from wheat and RCBr were sampled to analyse the expression of cytokinin-associated genes, including isopentenyltransferases (*IPT*s) and cytokinin oxidase/dehydrogenases (*CKX*s), using RT-qPCR.

INCYDE enhanced seed yield in RCBr when applied multiple times before flowering and under specific nitrogen conditions in growth room experiments, but neither INCYDE nor TDZ-K caused significant enhancement of yield following application to either wheat or barley in the field or in pot trials. However, INCYDE delayed senescence in barley in the pot trials. LC-MS/MS analyses revealed a strong peak in *t*Z cytokinins at four days after anthesis in wheat and barley, and an increase in the concentration of *c*ZOG following INCYDE treatment. Changes in expression of cytokinin biosynthesis (*IPT*) and degradation (*CKX*) gene family members occurred following INCYDE and TDZ-K treatment of both RCBr and wheat.

The enhancement in yield in RCBr following multiple INCYDE applications led to a feedback response model, which involved RCBr normalising cytokinin levels in response to changes in cytokinin homeostasis. The efficacy of the PGRs under optimal conditions in the field was minimal. Nonetheless, INCYDE showed an ability to delay senescence under conditions of imposed stress in the pot trials, and it was shown that the PGRs were able to affect cytokinin homeostasis by altering the expression of genes associated with cytokinin biosynthesis and degradation in both wheat and RCBr.

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Table of contents

List of figures

List of tables

Page

Abbreviations

°C	degrees Celsius
µL	microlitre
µM	micromolar
1°	primary leaf
2°	secondary leaf
3°	tertiary leaf
A_{647} and A_{664}	absorbance at 647 and 664 nm
ADP	adenosine 5'-diphosphate
AHK	arabidopsis histidine kinase
AMP	adenosine 5'-monophosphate
arabidopsis	*Arabidopsis thaliana*
ATP	adenosine 5'-triphosphate
B. napus	*Brassica napus*
B. oleracea	*Brassica oleracea*
B. rapa	*Brassica rapa*
B+R	base and riboside cytokinin
BA	N^6-benzylaminopurine
bp	base pair
cDNA	complementary deoxyribonucleic acid
CK	cytokinin
CKX	cytokinin oxidase/dehydrogenase
cm	centimetre
Control (-)	negative control
Control (+)	positive control
CPPU	*N*-(2-chloro-4-pyridyl)-*N*'-phenylurea
CRE1	cytokinin response 1
Ct	threshold cycle values
Cf	correction factor
cv.	cultivar
CWINV	cell wall invertase
*c*Z	*cis*-zeatin
*c*Z7G	*cis*-zeatin-7-*N*-glucoside
*c*Z9G	*cis*-zeatin-9-*N*-glucoside
*c*ZOG (enzyme)	*cis*-zeatin *O*-glucosyltransferase
*c*ZOG (cytokinin)	*cis*-zeatin-*O*-glucoside
*c*ZOX	*cis*-zeatin-*O*-xyloside
*c*ZR	*cis*-zeatin riboside
*c*ZRDP	*cis*-zeatin riboside-5'-diphosphate
*c*ZRMP	*cis*-zeatin riboside-5'-monophosphate
*c*ZROG	*cis*-zeatin riboside-*O*-glucoside
*c*ZRTP	*cis*-zeatin riboside-5'-triphosphate
d	day
daa	day after anthesis
daa/t	day after anthesis and treatment
daf	day after flowering
dat	day after treatment

DEPC	diethyl pyrocarbonate
DMAPP	dimethylallylpyrophosphate
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide
DTT	dithiothreitol
DW	dry weight
DZ or DHZ	dihydrozeatin
DZ7G	dihydrozeatin-7-glucoside
DZ9G	dihydrozeatin-9-glucoside
DZOG	dihydrozeatin-*O*-glucoside
DZR	dihydrozeatin riboside
DZRMP	dihydrozeatin riboside-5'-monophosphate
DZROG	dihydrozeatin riboside-*O*-glucoside
e.g.	exempli gratia (for example)
ELF	elongation factor 1
F	Tui Novatec Premium Fertiliser
FAD	flavin adenine dinucleotide
FW	fresh weight
g	grams
g	gravity
GAP	glyceraldehyde-3-phosphate dehydrogenase
gDNA	genomic DNA
GLU	*β*-glucosidase
GS	growth stage
GUS	*β*-glucuronidase
h	hours
ha	hectare
HATS	high affinity transport systems
HMBPP	4-hydroxy-3-methyl-2-(*E*)-butenyl diphosphate
HP	histidine phosphotransfer protein
IAA	indoleacetic acid
INCYDE	2-chloro-6-(3-methoxyphenyl)aminopurine
INV	invertases
iP	N^6-isopentenyladenine
iP7G	N^6-isopentenyladenosine-7-glucoside
iP9G	N^6-isopentenyladenosine-9-glucoside
iPR	N^6-isopentenyladenosine
iPRDP	N^6-isopentenyladenosine-5'-diphosphate
iPRMP	N^6-isopentenyladenosine-5'-monophosphate
iPRTP	N^6-isopentenyladenosine-5'-triphosphate
IPT	isopentenyltransferase
IRVI	Inverse Ratio Vegetative Index
kg	kilogram
Kin	kinetin
kpA	kilopascal
L	litre
LATS	low affinity transport systems
LOD	limit of detection

LOG	LONELY GUY
m	metre
mg	milligram
min	minute
mL	millilitre
mm	millimetre
mM	millimolar
mT	*meta*-topolin
N	nitrogen
n	sample number
NDVI	Normalised Difference Vegetative Index
ng	nanogram
nm	nanometre
nM	nanomolar
NS	not significant
NTC	no template control
oT	*ortho*-topolin
PCR	polymerase chain reaction
PGR	plant growth regulator
pmol	picomolar
P_{SAG12}	promoter of *SAG12*
P_{SARK}	promoter of senescence-associated receptor kinase (*SARK*)
RCBr	rapid cycling *Brassica rapa*
RNA	ribonucleic acid
RNAi	RNA interference
RNase	ribonuclease
RRA	type A response regulator
RRB	type B response regulator
RT-qPCR	reverse transcription quantitative polymerase chain reaction
S	Silwet L-77
s	second
SAM	shoot apical meristems
spp.	species
Tw	Tween 20
T	tonne
TCS	two component regulatory system
TDPG	uridine triphosphate glucose
TDZ	thidiazuron
TGW	thousand grain weight
tRNA	transfer RNA
*t*Z	*trans*-zeatin
*t*Z7G	*trans*-zeatin-7-*N*-glucoside
*t*Z9G	*trans*-zeatin-9-*N*-glucoside
*t*ZOG	*trans*-zeatin-*O*-glucoside
*t*ZOX	*trans*-zeatin-*O*-xyloside
*t*ZR	*trans*-zeatin riboside
*t*ZRDP	*trans*-zeatin riboside-5'-disphosphate
*t*ZRMP	*trans*-zeatin riboside-5'-monophosphate
*t*ZROG	*trans*-zeatin riboside-*O*-glucoside
*t*ZRTP	*trans*-zeatin riboside-5'-triphosphate

UDPG	uridine diphosphate glucose
UDPX	uridine diphosphate xylose
UV	ultra violet
V	volts
vs	versus
w/v	weight/volume
ZOG	*trans*-zeatin *O*-glucosyltransferase

Chapter 1
Introduction

1.1 Research background

Despite significant gains in food production, particularly due to the scientific and technological breakthroughs made during the Green Revolution (Evenson and Gollin, 2003), there are a growing number of challenges to maintaining and promoting food security. The global population is projected to reach over nine billion by 2050 with a large proportion of this growth occurring in the developing world (UN, 2017). Not only is the population increasing but so is food consumption *per capita* (Baldos and Hertel, 2014). Additionally, there is a general movement towards diets richer in protein. These factors are all contributing towards a need for significant increases in productivity and yield (Schmidhuber and Shetty, 2005; Gerbens-Leenes *et al*., 2010).

These trends are further complicated by additional challenges presented from environmental degradation, loss of biodiversity and climate change (Nellemann *et al*., 2009). Climate change will have far-reaching effects on agro-ecological conditions, land suitability and crop yields (Schmidhuber and Tubiello, 2007) and bring about additional uncertainties through its affects on disease pressure (IPCC, 2007), and the frequency of severe weather events including drought, flooding and cyclones (IPCC, 2001; IPCC, 2007). All of these factors further highlight the importance of global-level initiatives, research advances and innovation.

Agriculture in New Zealand makes up a significant proportion of its GDP and exports (Trading Economics, 2017). In the year ending in June 2016, around 50,000 hectares of both wheat and barley were harvested, with the Canterbury region making up a large proportion of this (Stats New Zealand, 2016). Given the restrictive regulations around growing genetically engineered crops in New Zealand, alternative approaches, including the use of synthetic plant growth regulators (PGRs) to enhance yield, continue to be investigated.

Novel plant growth regulators INCYDE and TDZ-K were acquired from a collaborator lab at Palacký University (Olomouc). INCYDE is reported to inhibit cytokinin oxidase/dehydrogenase (CKX)

(Zatloukal *et al*., 2008), an enzyme which catalyses the breakdown of the cytokinins, and is purported to enhance yield and growth in *Arabidopsis thaliana* (arabidopsis) and winter rapeseed (unpublished data, Palacký University). TDZ-K is purported to not inhibit root growth in arabidopsis and wheat, and inhibit senescence in wheat and barley leaves (United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript). INCYDE was also observed to enhance yield when plants were grown under 5 mM KNO_3 conditions in growth rooms (unpublished data, University of Canterbury). These preliminary experiments led to an investigation of the potential of each PGR to enhance yield and provide alleviation from environmental stress in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) in field and pot trials, and in the model species rapid cycling *Brassica rapa* (RCBr) under controlled growth room conditions.

Given that INCYDE is known to inhibit the degradation of cytokinins, an understanding of the role cytokinins play in the efficacy and effect of each compound was essential for this research. Samples extracted from the field and pot trials were used for gene expression studies and analyses of endogenous cytokinin content to elucidate underlying cytokinin regulation and physiology, and to provide an understanding of the mechanism of action of INCYDE and TDZ-K.

The Foundation for Arable Research (FAR) has a goal to increase the yield of wheat to 20 tonnes per hectare by 2020 (FAR, 2015). A PGR approach is particularly attractive to assist in this, because it is a practical and economically viable approach to yield enhancement that avoids the regulatory difficulties associated with transgenic crops. This research project was funded and supported by FAR, Callaghan Innovation and the Ministry of Business, Innovation and Employment (MBIE) and the University of Canterbury. Earlier work with growth room experiments was supported with funding from Ballance Agri-Nutrients.

1.2 Cytokinins

A compound capable of promoting cell division in the presence of the hormone auxin was isolated from autoclaved herring sperm DNA, and this compound was named kinetin (Miller *et al*., 1955). The effects of kinetin were studied extensively in tissue culture and when used with the auxin, indoleacetic acid (IAA), the shoot and root growth could be altered depending on the ratio of auxin to kinetin. The first naturally occuring cytokinin, zeatin, was isolated from maize endosperm (Letham, 1963). Subsequently, a number of natural and synthetic cytokinins have been discovered and synthesised.

Cytokinins are a functionally and spatio-temporally diverse plant hormone group involved in a broad range of developmental processes. These include shoot and root growth (Werner *et al*., 2001; 2003; Brenner and Schmülling, 2012), branching (Müller and Leyser, 2011), meristem development (Su *et al*., 2011), flower promotion and floral development (Bartrina *et al*., 2011; D'Aloia *et al*., 2011), seed development (Riefler *et al*., 2006; Jameson and Song, 2016) and seed germination (Miransari and Smith, 2014). Cytokinins are also extensively involved in nitrogen metabolism (Takei *et al*., 2004a; Shtratnikova *et al*., 2015), signal transduction (Jones *et al*., 2010), the plant response to biotic and abiotic stress (O'Brien and Benková, 2013) and senescence (Gan and Amasino, 1996) amongst many other processes.

1.2.1 Cytokinin structure

Cytokinins are categorised into three groups. These include naturally occurring adenine derivatives of cytokinin, which contain various N^6 substituents (Mok and Mok, 2001; Spíchal, 2012). Adenine cytokinins include the isoprenoid cytokinins *trans*-zeatin (*t*Z), *cis*-zeatin (*c*Z), N^6-isopentenyladenine (iP) and dihydrozeatin (DHZ or DZ) (**Figure 1.1**). The second group of adenine derivatives is the aromatic cytokinins, each of which contain an aromatic side chain with substitutions at different positions. Aromatic cytokinins include the synthetic kinetin (Kin), the naturally occurring N^6-benzylaminopurine (BA), and its hydroxylated derivatives known as topolins. The third group includes the synthetic phenylurea cytokinins, which includes the PGRs CPPU (forchlorfenuron) and thidiazuron (TDZ).

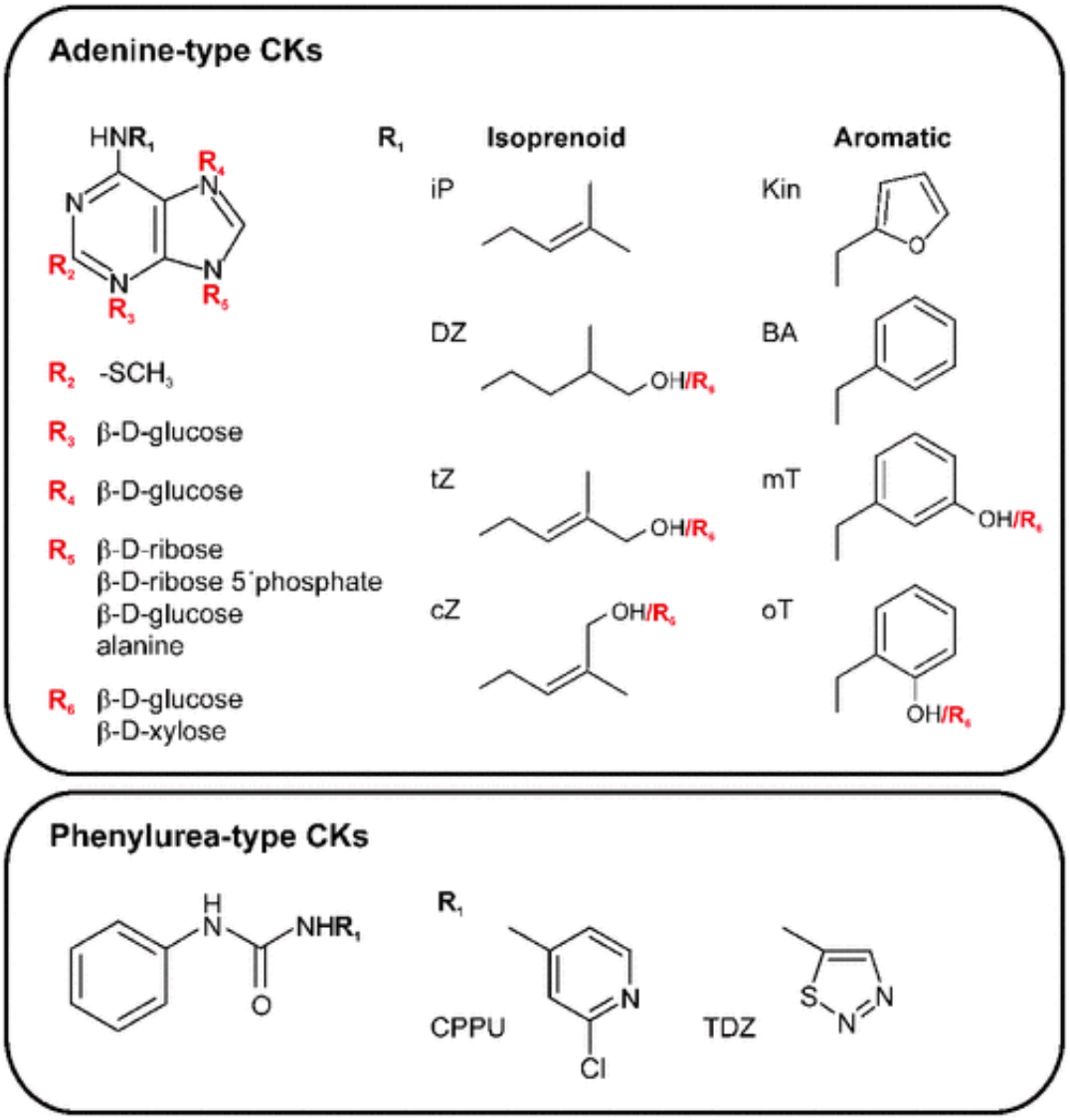

Figure 1.1 The chemical structure of adenine-type cytokinins and phenylurea-type cytokinins. The R1 side chain denotes the side chain that determines the cytokinin, while R2 to R6 positions indicate the conjugate, which results from metabolic interconversions. These interconversions are described in Figure 1.2. The full name of cytokinins described with abbreviations above include: iP (N^6-isopentenyladenine); DZ (dihydrozeatin); *t*Z (*trans*-zeatin); *c*Z (*cis*-zeatin); Kin (kinetin); BA (N^6-benzylaminopurine); mT (*meta*-topolin); oT (*ortho*-topolin); CPPU (*N*-(2-chloro-4-pyridyl)-*N*'-phenylurea); TDZ (thidiazuron). Image taken from Spíchal (2012), published with permission.

Cytokinins can exist in different forms, including an active form, (e.g. zeatin), as a nucleoside (e.g. zeatin riboside) and as a nucleotide (e.g. zeatin ribotide) (Spíchal, 2012). A number of enzymes and pathways have been established that allow for the biosynthesis, breakdown, inactivation, deactivation, modification, and interconversion between different cytokinin types and forms (**Figure 1.2**). The nucleotide form is the form first produced by biosynthesis, and can be converted directly to the active nucleobase by riboside 5'-monophosphate phosphohydrolase, an enzyme encoded by LONELY GUY (LOG) (Kuroha *et al*., 2009; Tokunaga *et al*., 2012). While still in the nucleotide form, iP nucleotides can be converted to *t*Z nucleotides by hydroxylation of prenyl side chain, which is catalysed by cytochrome P450 monooxygenases (Takei *et al*., 2004b).

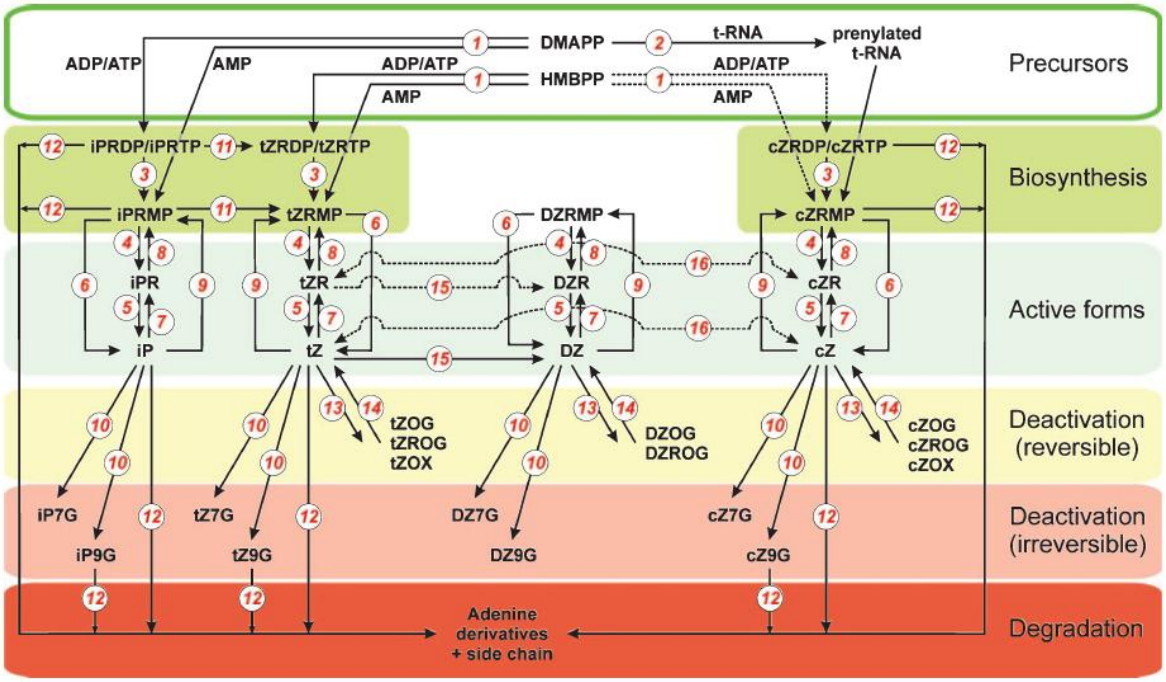

Figure 1.2 A scheme of cytokinin biosynthesis, degradation and the known interconversions. Red numbers indicate enzymes involved at each step. Dashed lines indicate pathways that have not been sufficiently substantiated. The enzymes represented by each red number are: 1) adenylate isopentenyltransferase (EC 2.5.1.27); 2) tRNA-specific isopentenyltransferase (EC2.5.1.8); 3) phosphatase (EC 3.1.3.1); 4) 5'-ribonucleotide phosphohydrolase (EC 3.1.3.5); 5) adenosine nucleosidase (EC 3.2.2.7); 6) Cytokinin phosphoribohydrolase 'Lonely guy'; 7) purine nucleoside phosphorylase (EC 2.4.2.1); 8) adenosine kinase (EC 2.7.1.20); 9) adenine phosphoribosyltransferase (EC 2.4.2.7); 10) *N*-glucosyl transferase (EC 2.4.1.118); 11) cytochrome P450 mono-oxygenase; 12) cytokinin dehydrogenase (EC 1.5.99.12); 13) zeatin-*O*-glucosyltransferase, which is either *trans*-zeatin-specific (EC 2.4.1.203) or *cis*-zeatin-specific (EC 2.4.1.215), utilising xylose instead of glucose (EC 2.4.2.40); 14) *β*-glucosidase (EC 3.2.1.21); 15) zeatin reductase (EC 1.3.1.69); 16) zeatin isomerase. Abbreviations include: DMAPP, dimethylallylpyrophosphate; HMBPP, 4-hydroxy-3-methyl-2-(*E*)-butenyl diphosphate; iPRDP, N^6-isopentenyladenosine-5'-diphosphate; iPRTP, N^6-isopentenyladenosine-5'-triphosphate; iPRMP, N^6-isopentenyladenosine-5'-monophosphate; iPR, N^6-isopentenyladenosine; iP7G, N^6-isopentenyladenosine-7-glucoside; iP9G, N^6-isopentenyladenosine-9-glucoside, and the equivalents for *t*Z, DZ and *c*Z; *t*ZOG, *trans*-zeatin-*O*-glucoside; *t*ZROG, *trans*-zeatin-*O*-glucoside riboside and the equivalents for DZ and *c*Z; *t*ZOX, *trans*-zeatin-*O*-xyloside; *c*ZOX, *cis*-zeatin-*O*-xyloside. NOTE: riboside cytokinins are no longer considered to be active forms (Lomin *et al*., 2015). Image taken from Spíchal (2012). Published with permission.

1.2.2 Cytokinin biosynthesis

Isopentenyltransferases (IPTs) are the enzyme family responsible for the biosynthesis of cytokinin (Kakimoto, 2001; Takei *et al*., 2001; Frébort *et al*., 2011). A family of isopentenyltransferase genes (IPTs) encoding for the enzyme, were discovered in arabidopsis (Kakimoto, 2001; Takei *et al*., 2001). The *AtIPT* gene family members are divided into two groups: those coding for IPTs that participate in a *de novo* biosynthesis pathway (which include *AtIPT1*, *AtIPT3* to *8*), and a second group involving putative tRNA IPTs (which include *AtIPT2* and *AtIPT9*). The biosynthetic function of these genes were confirmed with *IPT*-overexpression experiments in a transformed callus, which in the presence of auxin regenerated shoots (Kakimoto *et al*., 2001), a phenotype that aligns with exogenous cytokinin application experiments (Skoog and Miller, 1957). Using a *β*-glucuronidase (GUS) gene fused to *AtIPT* the promoter region, or a promoter plus the entire *IPT* coding region, insight was provided on the expression patterns of *AtIPT*s and revealed that expression was tissue-specific. For example, *AtIPT1::GUS* was expressed in xylem precursor cells (in the root cambium), ovules and immature seeds, *AtIPT3::GUS* was expressed in the phloem, *AtIPT4::GUS* and *AtIPT8::GUS* in the chalazal endosperm of immature seeds, while *AtIPT7::GUS* was expressed in leaf trichomes (Miyawaki *et al*., 2004).

The adenylate IPTs (EC 2.5.1.27) catalyse a *de novo* biosynthesis pathway, which is the main contributor towards the overall pool of cytokinins (**Figure 1.2**). The adenylate IPTs isoprenylate adenosine phosphates (ADP/ATP) with an isoprenoid moiety, via the N^6 position (Miyawaki *et al*., 2004; Spíchal, 2012). The isopentenyl moiety dimethylallyl diphosphate (DMAPP) is used to produce iP-type cytokinins. Plant IPTs have a preference for using ATP and ADP and, in contrast to Agrobacterial IPTs (Ueda *et al*., 2012), DMAPP is the preferred donor to form precursors N^6-isopentenyladenosine-5'-triphosphate (iPTP) or iPDP.

The turnover of transfer RNA was first considered a cytokinin source because of the presence of a isopentenyladenosine residue, adjacent to a specific tRNA anticodon. This second group of IPTs, known as tRNA IPTs (EC 2.5.1.8), produces *c*Z cytokinins and involves the prenylation of tRNA by the addition of DMAPP to an adenine residue (Miyawaki *et al*., 2004; Sakakibara *et al*., 2006). Expression studies have revealed that tRNA *IPT*s are constitutively expressed in some species (Miyawaki *et al*., 2004; Song *et al*., 2015).

1.2.3 Cytokinin perception and signal transduction

Cytokinin signal transduction involves a two component regulatory system (TCS), that consists of a sensor and response regulators interacting via a phosphorelay system (Mizuno, 2005; Nishijima, 2012). The receptor component of this system involves histidine protein kinases (HKs). Several of these have been identified in arabidopsis including cytokinin response 1 (CRE1) (Inoue *et al*., 2001) and arabidopsis histidine kinase 2, 3, and 4 (AHK2, 3 and 4) (Suzuki *et al*., 2001). Receptors CRE1 and AHK4 were found to be identical and consequentially referred to as CRE1/AHK4. Recently, only cytokinin bases (such as *t*Z), have been confirmed to be able to bind to cytokinin receptors, while the riboside forms are unable to (Lomin *et al*., 2015).

Once the receptors have been activated by a free base cytokinin, this results in kinase activity which autophosphorylates the histidine residue within the receptor transmitter domain of the HKs. The phosphate is then released onto the receiver domain via the aspartate residue, resulting in a subsequent downstream phosphorylation of histidine phosphotransfer proteins (HPs) (Nishijima, 2012). These phosphorylated HPs migrate to the nucleus and phosphorylate response regulator B via the aspartate residue (Hwang and Sheen 2001; Werner and Schmülling, 2009).

Response regulators (RRs) are divided into two structurally different groups: Type A RRs (RRA) and Type B RRs (RRB) (D'Agostino and Kieber 1999; Imamura *et al*., 1999). Both groups compete for phosphorylation from the histidine phosphotransfer proteins (Gupta and Rashotte, 2012). Type A RRs lack a GARP DNA-binding domain found in RRBs (Sakai *et al*., 2000; Hosoda, *et al*., 2002) and RRAs only have receiver domains. Conversely, RRBs which have both receivers and outputs (Nguyen *et al*., 2016). Type A RRs are considered negative regulators of cytokinin signalling, while RRBs are considered positive regulators (To *et al*., 2004; Mason *et al*., 2005). While Type B RRs act as transcription factors that regulate the expression of specific cytokinin response genes, RRAs are induced and regulated in part by RRB (Hwang and Sheen, 2001; Sakai *et al*., 2001), and when accumulated can act to repress cytokinin signalling and decrease the sensitivity of the response to cytokinin (Rashotte *et al*., 2003). When RRAs have reduced functionality, the cytokinin response can be enhanced (To *et al*., 2004), further establishing RRAs function as a negative feedback loop in the primary cytokinin signal transduction pathway (Hwang and Sheen, 2001; Lee *et al*., 2007; Spíchal, 2012; Nisler *et al*., 2016).

There exist antagonists of cytokinin receptors including PI-55. The compound PI-55 acts as a partial receptor antagonist and while it is blocks CRE1/AHK4, it can weakly activate AHK3 at high concentrations (Spíchal *et al*., 2009). Treatment with this antagonist results in phenotypes associated with a reduced cytokinin status including the promotion of root growth (Spíchal *et al*., 2009) and provide plants with resistance to stress from heavy metals (Gemrotová *et al*., 2013). This resistance to abiotic stress aligns with experiments where decreased cytokinin perception, by reduced sensitivity of cytokinin receptors allows plants to handle various abiotic stress including drought stress (Tran *et al*., 2007). Using receptor antagonists has been identified as a useful strategy for manipulating endogenous cytokinin levels (Gemrotová *et al*., 2013).

1.2.4 Metabolism

1.2.4.1 Cytokinin degradation

Cytokinins can be inactivated by conjugation or degradation. Cytokinin oxidase/dehydrogenase (EC.1.5.99.12) or CKX, is a group of enzymes responsible for degradation, and it does this by catalysing the irreversible cleavage of the side chain from cytokinins to produce an adenine and aldehyde (McGaw *et al*., 1983; Galuszka *et al*., 2000) (**Figure 1.2**). This reaction involves the use of a flavin adenine dinucleotide (FAD) cofactor (Bilyeu *et al*., 2001). At first CKX was considered an oxidase, and referred to as CKO, because of its capacity to use oxygen as an electron acceptor (Hare and van Staden, 1994a). It was later discovered that it was able to utilise a number of other electron acceptors (under anaerobic conditions), and was reclassified as a dehydrogenase (EC 1.5.99.12) and subsequently referred to as cytokinin oxidase/dehydrogenase to reflect this (Galuszka *et al*., 2001; Frébort *et al*., 2002).

A number of different isoforms of CKX exist, and these collectively use a broad range of substrates including isoprenoid free base cytokinins iP, *t*Z and *c*Z, and their riboside forms (Gajdošová *et al*., 2011). Cytokinin oxidase/dehydrogenases are also able to cleave nucleotide forms, *N*-9 glucosides (Kowalska *et al*., 2010) and aromatic cytokinins (Galuszka *et al*., 2007). Cytokinin oxidase/dehydrogenase isoforms show specificity to substrates. In *Zea mays* for example, ZmCKX1 has a preference for oxidising cytokinins in the base and riboside forms, with the strongest preference towards iP (Bilyeu *et al*., 2001; Kopečný *et al*., 2005). Conversely, isoforms ZmCKX2, 3, 4a, 4b and 5 have a strong preference for cytokinins N^6-isopentenyladenosine-9-glucoside (iP9G) and N^6-(Δ^2-isopentenyl)adenosine-5′-monophosphate (iPRMP) (Nisler *et al*., 2016). In addition to exhibiting substrate specificity, CKXs are encoded by multigene families (Schmülling *et al*., 2003), in

arabidopsis, seven have been identified, while 11 have been identified in rice and 13 in maize (Mameaux *et al*., 2012; Zalabák *et al*., 2014). CKX gene family members show differential spatio-temporally expression patterns and exist in a number subcellular locations (Morris *et al*., 1999; Šmehilová *et al*., 2009; Vyroubalová *et al*., 2009; Gu *et al*., 2010; Zalabák *et al*., 2014; Nisler *et al*., 2016).

Cytokinin oxidase/dehydrogenases have been identified as an important biotechnological targets for increasing endogenous cytokinin through inhibition of its activity (Gemrotová *et al*., 2013; Nisler *et al*., 2016). Several compounds have been identified that inhibit CKX. These include urea compounds including diphenylureas thidiazuron and CPPU (Chatfield and Armstrong 1986; Burch and Horgan 1989; Laloue and Fox 1989; Hare and van Staden 1994b; Nisler *et al*., 2016). These compounds are PGRs that are already used widely in horticulture (Arima *et al*., 1994). Other compounds that are capable of CKX inhibition include anilinopurine derivatives such as 2-chloro-6-anilinopurine (Zatloukal *et al*., 2008) and suicide substrates such as N^6-(buta-2,3-dienyl)adenine (Suttle and Mornet, 2005; Kopečný *et al*., 2008).

1.2.4.2 *N*-glucosylation

The purine ring of cytokinins can be modified by *N*-glucosylation of the *N*-3, *N*-7 and *N*-9 positions to form conjugates (Hou *et al*., 2004). This is catalysed by glucosyltransferases (EC 2.4.1.118) which utilise uridine diphosphate glucose (UDPG) and uridine triphosphate glucose (UDPG) as donors (**Figure 1.2**). *N*-glucosylation produces *N*-7 glucosides including *trans*-zeatin-7-*N*-glucoside (*t*Z7G) and *cis*-zeatin-7-*N*-glucoside (*c*Z7G), and *N*-9 glucosides including *trans*-zeatin-9-*N*-glucoside (*t*Z9G) and *cis*-zeatin-9-*N*-glucoside (*c*Z9G), and this inactivation is irreversible (Mok and Mok, 2001). The *N*-glucosides can subsequently be degraded by CKX (Galuszka *et al*., 2007; Kowalska *et al*., 2010).

1.2.4.3 *O*-glucosylation

Cytokinins can also be inactivated through *O*-glucosylation of the N^6-side chain hydroxyl group. This is carried out by *trans*-zeatin *O*-glucosyltransferase (ZOG) (EC 2.4.1.203) which catalyses the *O*-glucosylation of *t*Z into *O*-glucoside *t*ZOG, and *cis*-zeatin *O*-glucosyltransferase (*c*ZOG) (EC 2.4.1.215) which *O*-glucosylates *c*Z to its *O*-glucoside form *c*ZOG (**Figure 1.2**) (Jin *et al*., 2013). While *c*ZOG only recognises *c*Z and uses uridine diphosphate glucose (UDPG) as a substrate for conjugation, ZOG can recognise *t*Z and DHZ and utilises substrates UDPG and uridine diphosphate xylose (UDPX) as donor substrates respectively for each cytokinin (Mok and Mok, 2001). Cytokinin

O-glucosides can be converted back to active cytokinin forms by the action of *β*-glucosidase (Werbrouck *et al*., 1996). Their ability to be reconverted back based on the requirements of the plant, along with the resistance of these forms to CKX cleavage (Galuszka *et al*., 2007), indicates their role as storage cytokinins (Veach *et al*., 2003; Kiran *et al*., 2006) and provides a point of homeostatic control over cytokinin activity (Mok and Mok, 2001). The enzyme ZOG was first isolated from *Phaseolus lunatus* (Dixon *et al*., 1989), and the gene subsequently identified (Martin *et al*., 1999). Genes for *cZOG* were later identified in maize (Martin *et al*., 2001; Veach *et al*., 2003).

1.2.4.4 Cytokinin reactivation

β-glucosidases (GLU) (EC 3.2.1.21) are a large group of enzymes that catalyse the hydrolysis of glycosidic links in *O*-glucosides and N^3-glucosides, converting them into their free forms, and producing glucose as a secondary product (Smith and van Staden, 1978; Brzobohatý *et al*., 1993; Jameson, 1994) (**Figure 1.2**). They have an important role in ensuring a continuous supply of active cytokinins from the inactive storage forms (Kiran *et al*., 2006). Different *β*-glucosidases are involved in a number of developmental processes including seed development (Leah *et al*., 1995), and abiotic stress response (Baba *et al*., 2017) and secondary metabolism (Cairns and Esen, 2010). Experiments with vacuole-targeted overexpression of *β*-glucosidase *BglB* in tobacco, has been shown to increase the level of active CKs and result in phenotypes associated with cytokinin enhancement including promoting stem growth, increasing plant biomass and earlier flowering (Nguyen *et al*., 2015).

1.2.5 Cytokinins and yield

Cytokinins play a prominent role in seed development and yield determination (Riefler *et al*., 2006; Jameson and Song, 2016, and references therein). In arabidopsis *AtIPT1*, *AtIPT4* and *AtIPT8* have been identified to have a role in seed development, with each showing expression in developing seeds (Miyawaki *et al*., 2004; Belmonte *et al*., 2013). In cereals, a transient increase in cytokinin has been observed in developing seeds within days post-anthesis (Jameson *et al*., 1982; Morris *et al*., 1993; Dietrich *et al*., 1995; Banowetz *et al*., 1999a; 1999b) and in wheat there is a peak in the expression of *TaIPT*s and *TaCKX*s (Song *et al*., 2012) and in barley an elevated expression of some *HvCKX*s post-anthesis (Zalewski *et al*., 2014) and a strong presence of *c*Z early in development as the kernel develops (Powell *et al*., 2013).

The cytokinin peak is known to coincide with endosperm proliferation in developing seeds (Dietrich *et al*., 1995; Banowetz *et al*., 1999b; Brugière *et al*., 2008), further highlighting the importance of

cytokinin in seed development. An elevation of cytokinin has also been observed in other plant groups including legumes (Emery *et al*., 2000).

In order to enhance yield, a number of approaches have been implemented to manipulate endogenous cytokinin in plants (Jameson and Song, 2016, and references therein). Increasing the concentration of endogenous cytokinin by applying cytokinin exogenously is one approach that has been studied extensively in a number of species (Koprna *et al*., 2016). There is evidence that exogenous application of cytokinins such as BA or kinetin has successfully affected components of yield in cereals, including in wheat (Wang *et al*., 2001; Gupta *et al*., 2003), barley (Williams and Cartwright, 1980; Hosseini *et al*., 2008), rice (Yang *et al*., 2002) and maize (Amin *et al*., 2007) under various conditions. There are, however, limitations and complexities to applying cytokinins in field trials. The response of plants can be varied, inconsistent and dependent on a number of factors including the method of application, cytokinin concentration, development stage targeted, growth conditions (including the presence of stress) and the species used (Koprna *et al*., 2016), and this limits the efficacy of this approach.

In legumes, for example, cytokinin application has resulted in a number of different growth responses under different conditions (Cho *et al*., 2002; Liu *et al*., 2004; Nonokawa *et al*., 2007), but even when yield enhancements have been observed, there were sometimes difficulties repeating these enhancements in the field (Nagel *et al*., 2001). The responses to cytokinins can even differ based on what parts of the plant are targeted (Hosseini *et al*., 2008). Cytokinins have most commonly been applied by spraying or irrigation (Koprna *et al*., 2016). Although there has been some success with altering yield by injecting cytokinin directly into organs of the plant (Warrier *et al*., 1987; Sivakumar *et al*., 2001; Gupta *et al*., 2003), this approach is clearly impractical in the field. Other approaches to enhancing yield by exogenous application have focused on directly altering tillering with the aim of increasing the number of productive tillers (Langer *et al*., 1973; Harrison and Kaufman, 1980; Koprna *et al*., 2016).

Another approach has been to use transgenics by modifying the expression of *IPT*s and *CKX*s. This approach has its own difficulties, including overproducing cytokinin following *IPT*-overexpression, which can result in developmental abnormalities (Guo and Gan, 2014, and references therein). This suggests that transgenic approaches need to precisely and modestly manipulate cytokinin levels in order to enhance yield and growth (Jameson and Song, 2016). Targeting cytokinin biosynthesis or degradation genes in the shoot apical meristem (SAM) has been identified as a way to enhance seed

number (Jameson and Song, 2016). In Ashikari *et al*. (2005) grain number was identified as being controlled by *OsCKX2*. A reduction of *Osckx2* expression resulted in an accumulation of cytokinin in the inflorescence meristem, and this led to increases in the grain number. The importance of *OsCKX2* in yield determination was confirmed with transgenic experiments: transgenic overexpression of *OsCKX2* reduced grain number while a reduction in *OsCKX2* expression with antisense cDNA led to an enhancement in seed number (Ashikari *et al*., 2005). In arabidopsis, a double mutant for *CKX3* and *CKX5* (*Atckx3ckx5*), both genes which are expressed in the meristem, resulted in an increase in the size of floral organs, the number of siliques, number of seeds per silique and the overall seed number (Bartrina *et al*., 2011). The enhancement in yield observed in Bartrina *et al*. (2011) occurred in the absence of an increase in the strength of the source (the carbon-fixing parts of the plant), and was instead dependent on the strength of the sink (the parts supplied with carbon). This suggests the importance of the sink strength in determining yield, further highlighting the importance of targeting the reproductive meristems, which determine the strength of this sink. The ideal approach to yield enhancement will depend on a knowledge of whether a plant is source-limited or sink-limited (Jameson and Song, 2016).

Although an inverse relationship is well-known to exist between seed number and the seed size (Paul-Victor and Turnbull, 2009; Van Daele *et al*., 2012), this relationship does not necessarily apply when cytokinin levels are enhanced (Jameson and Song, 2016, and references therein). The seed weight has been enhanced by manipulating cytokinin content in the developing seed using seed-specific promoters. This has been observed in transgenic tobacco experiments using ectopic *IPT*-overexpression with embryo-specific (Ma *et al*., 2002) and endosperm-specific promoters (Daskalova *et al*., 2007) where seed weight was enhanced in each case. In Ma *et al*. (2008), increases in seed weight and in the content of protein and carbohydrate were observed when overexpressing *IPT* under the control of a seed-specific lectin in tobacco, without resulting in other growth abnormalities. In wheat, *TaCKX6-D1* variants have been observed to affect the thousand grain weight, but not grain number, with haplotypes with decreased *TaCKX6-D1* expression having greater grain weights (Zhang *et al*., 2012). Other approaches to enhancing yield have included the use of RNAi silencing of barley grain-expressed CKXs (Zalewski *et al*., 2010; 2012; 2014), or enhancing the yield in cotton by moderately increasing cytokinin across the plant using a constitutively-expressed RNAi *CKX* construct (Zhao *et al*., 2015). These transgenic experiments and the injector experiment mentioned earlier, suggest that seed set, pod set and flowering are all cytokinin-limited. Zhao *et al*. (2015) has suggested that CKX was a softer regulator of cytokinin levels in comparison to *IPT*, and therefore a good target for yield enhancement.

Given the varied and often inconsistent effect of exogenous cytokinin application, it has been suggested that enhancing endogenous cytokinin using CKX-inhibiting compounds might be a more effective strategy for inducing desired growth effects (Gemrotová *et al*., 2013; Nisler *et al*., 2016). There are important commercial applications of CKX-inhibiting PGRs TDZ (Arndt *et al*., 1976) and CPPU (Hayata *et al*., 1995) and, with the synthesis of novel CKX-inhibiting compounds (Zatloukal *et al*., 2008), this approach has been adopted in this thesis.

1.2.6 Cytokinins and nitrogen

Plants require nitrogen in the form of nitrate (NO_3^-), ammonium (NH_4^+) and urea for growth and development (Dechorgnat *et al*., 2010), often requiring a mixture of nitrogen sources simultaneously for optimal growth (Cao *et al*., 1993; Shtratnikova *et al*., 2015). Plants adapt to the nitrogen source and availability of the immediate environment, and part of this adaption involves activating different nitrogen uptake systems. When nitrate concentrations are > 1 mM, plants utilise low affinity transport systems (LATS) (Touraine and Glass, 1997; Glass and Kotur, 2013). When nitrate concentrations are very low (< 1 mM), plants utilise high affinity transport systems (HATS) (Krapp *et al*., 2014). Ammonium is taken up separately by ammonium-specific transporters (Engelsberger *et al*., 2012).

Nitrate supply is well-known to correlate with cytokinin production (Sattelmacher and Marschner, 1978; Samuelson and Larsson, 1993), and there are several pieces of evidence to suggest that cytokinin acts as a long distance root-to-shoot signal to communicate nitrogen availability in plants (Sakakibara *et al*., 2006; Kiba *et al*., 2011). This includes evidence of an increase in the cytokinin content of xylem sap in maize following nitrate supplementation (Takei *et al*., 2001). Additionally, symptoms of nitrate deficiency throughout the plant can be reduced by the addition of cytokinin (Shtratnikova *et al*., 2015), supporting the idea of cytokinin being the limiting factor and signal. Cytokinin does not just act as a signal for nitrogen supplementation, but also acts as a signal for the nitrogen status itself. This was shown in experiments with arabidopsis seedlings, where a higher level of cytokinin was found when plants were provided with high nitrate conditions, compared to when plants were grown under low nitrate conditions (Kiba *et al*., 2011).

The transduction from perception of nitrogen into a cytokinin signal occurs via increases in *IPT* expression (Cline *et al*., 2006). The expression of *AtIPT3* is known to be directly induced by the presence of nitrate (Miyawaki *et al*., 2004; Takei *et al*., 2004a; Kamada-Nobusada *et al*., 2013), and

its expression is likely to provide the long distance cytokinin signal for communicating nitrogen availability to the plant. The overlap of expression of *AtIPT3* with the expression of root-based nitrate transporter suggests that mediation occurs at the level of a transporter (Kiba *et al*., 2011). These transporters can act as both a nitrogen transporter and nitrogen sensor (Ho *et al*., 2009; Wang *et al*., 2009).

1.2.7 Senescence and stress

Senescence is an active process involving controlled degradation of macromolecules (lipids, proteins, RNA) and the remobilisation and recycling of carbon and nitrogen into tissues including younger, active photosynthesising leaves and developing seeds (Gan and Amasino, 1997; Jordi *et al*., 2000; Roitsch and Ehneß, 2000; Guo and Gan, 2014). In leaves, senescence is characterised by yellowing and is the final developmental stage following leaf expansion and maturation (Guiboileau *et al*., 2010). Control over senescence is complex (Fischer, 2012), and senescence can be induced prematurely and accelerated by stress conditions, including nutrient deficiency or drought conditions (Pourtau *et al*., 2004; Rivero *et al*., 2009).

Cytokinins have an important role in drought-induced senescence, and the progression of drought stress itself is known to correlate with a reduction (or hastened decline) of the concentration of cytokinins (Yang *et al*., 2001; Kudoyarova *et al*., 2007; Ghanem *et al*., 2008; Havlová *et al*., 2008; Le *et al*., 2012). Conversely, enhancement of cytokinin such as through exogenous application or transgenic *IPT*-overexpression is known to preserve chlorophyll, retard or delay senescence and remobilise nutrients (Clarke *et al*., 1994; Gan and Amasino, 1995; Rivero *et al*., 2009; Guo and Gan, 2014).

The most effective transgenic approach to delaying senescence has been with using autoregulatory 'stay-green' systems P_{SAG}-*IPT* and P_{SARK}-*IPT* which are under the control of senescence-induced promoters P_{SAG12} or P_{SARK} (Hajouj *et al*., 2000). When tissues undergo senescence, these promoters induce the biosynthesis of cytokinin and this local increase in cytokinin reduces the rate of senescence. This elevation of cytokinin then acts as a feedback to inhibit continued expression of the autoregulatory system. This system prevents excess cytokinin production which might otherwise result in abnormal growth. Ultimately, these autoregulatory transgenic plants reduce yield loss and ameliorate symptoms induced by stress conditions (Guo and Gan, 2014, and references therein). By delaying senescence, this can increase the period of time that leaves can continue to photosynthesise,

which can increase the production of photosynthates, which can increase yield under drought conditions in rice (Peleg *et al*., 2011) and, in some cases, under both drought and well-watered conditions such as in *B. napus* (Kant *et al*., 2015). Compounds that inhibit senescence are also research targets that might offer yield enhancements or an amelioration from the effects of various stresses.

Cytokinins are known to have a complex role in abiotic stress response (Zwack *et al*., 2015, and references therein), part of this role during stress response involves cross-talk with other hormones (O'Brien *et al*., 2013). Exogenous cytokinin application has been known to have mixed effects of stress resistance, with application resulting in bean plants becoming more susceptible to salt stress (Kirkham *et al*., 1974), while cytokinin application provided some resistance to the effects of salt stress in wheat seedlings (Naqvi *et al*., 1982). Cytokinins also have an important role in disease development such as with clubroot (Siemens *et al*., 2006; Malinowski *et al*., 2016), with the progress of disease even aided by the pathogenic synthesis of cytokinins (Choi *et al*., 2011).

1.2.7.1 Invertases

Sucrose exits phloem-based sieve elements via a sucrose transporter before being subject to invertase activity. Invertases (INVs) (EC 3.2.1.26), are enzymes that catalyse the irreversible hydrolysis of sucrose into monosaccharides fructose and glucose, which are then transported by hexose transporters to sink cells (Roitsch and González, 2004). Invertases are categorised by their subcellular locations: the vacuole, cytosol or cell wall (Canam *et al*., 2008). Invertases have an important role in source-sink dynamics, and through their activity are able to determine the sink strength of local tissues and the mobilisation of nutrients throughout the plant (Balibrea Lara *et al*., 2004). Invertase activity is linked to cytokinin activity, and can be stimulated by cytokinins (Ehneß and Roitsch, 1997; Godt and Roitsch, 1997). Invertases play an important role as mediators of cytokinin-induced delay of senescence, with invertase activity correlating with a delay in senescence, and even being capable of substituting for cytokinin in delaying senescence (Balibrea Lara *et al*., 2004).

1.3 Plant growth regulators

1.3.1 INCYDE

INCYDE (INhibitor of CYtokinin DEhydrogenase, 2-chloro-6-(3-methoxyphenyl)aminopurine) is a substituted 6-anilinopurine derivative (**Figure 1.3A**). It has been shown to be a potent inhibitor of CKX (Spichal *et al*., ACPD conference 2009, IPGSA conference 2010; Nisler *et al*., 2012, unpublished manuscript); it has an IC_{50} of 1.9 µM (where IC_{50} is the concentration required for 50% inhibition of AtCKX2), and shown to be able to bind to cytokinin receptors in binding assays with an EC_{50} (CRE1/AHK4) of 7 µM and an EC_{50} (AHK3) of 21 µM (where EC_{50} is the concentration required for 50% of the activation response) (Zatloukal *et al*., 2008). Its ability to inhibit CKX is proposed to be an effective strategy for modifying endogenous cytokinin (Gemrotová *et al*., 2013; Nisler *et al*., 2016).

There are reports of increases in growth and yield following 10 and 50 µM INCYDE spray applications (under field conditions) with winter rapeseed (*B. napus* L.), including increases in branching, the number of siliques and the seed yield overall, and following four 10 µM applications targeting arabidopsis early in development, increases in the shoot growth, branching, biomass, seeds per silique and the seed yield overall (unpublished data, Palacký University; personal communication, May 22, 2012).

The promotion of floral growth is also evident following foliar spraying 10 nM INCYDE, with an increase in the number of flowers observed in tomato (Aremu *et al*., 2014) and increases in floral organ size following 10 µM with arabidopsis and 50 µM with *B. napus* (unpublished data, Palacký University). Conversely, dose-dependent inhibition of shoot and/or root growth has also been reported in *Rumex crispus* and *Bulbine natalensis* seedlings when seeds were provided with solutions containing 10 µM INCYDE (Gemrotová *et al*., 2013), and with arabidopsis grown in 10 to 100 nM in solution (in contrast to 1 nM INCYDE) (unpublished data, Palacký University), indicating a biphasic response. In micropropagated *Eucomis autumnalis*, a dose-dependent decrease in shoot length was observed when INCYDE was supplied at concentrations between 0.01 to 10 µM, while an enhancement of shoot number was observed in the presence of BA in media supplied with INCYDE (Aremu *et al*., 2015).

INCYDE can also alleviate symptoms and provide resistance to biotic and abiotic stresses. This includes reducing necrotic senescence symptoms following *Verticillium longisporum* infection in arabidopsis plants sprayed every third day (from four to 24 days after infection) with 10 µM INCYDE (Reusche *et al*., 2013), providing alleviation to the effects of salt stress in tomatoes following 10 nM spray or drenching (Aremu *et al*., 2014), and reducing the effects of cadmium stress in *Rumex crispus* L. and *Bulbine natalensis* seedlings when supplied at 100 nM (Gemrotová *et al*., 2013). INCYDE has been reported to enhance antioxidant activity in tomato following 10 nM spray or drenching (Aremu *et al*., 2014) and when applied at 100 µM in combination with BA (compared to BA alone) increased the concentration of *O*-glucosides in aerial parts of "Williams" banana plantlets (Aremu *et al*., 2012).

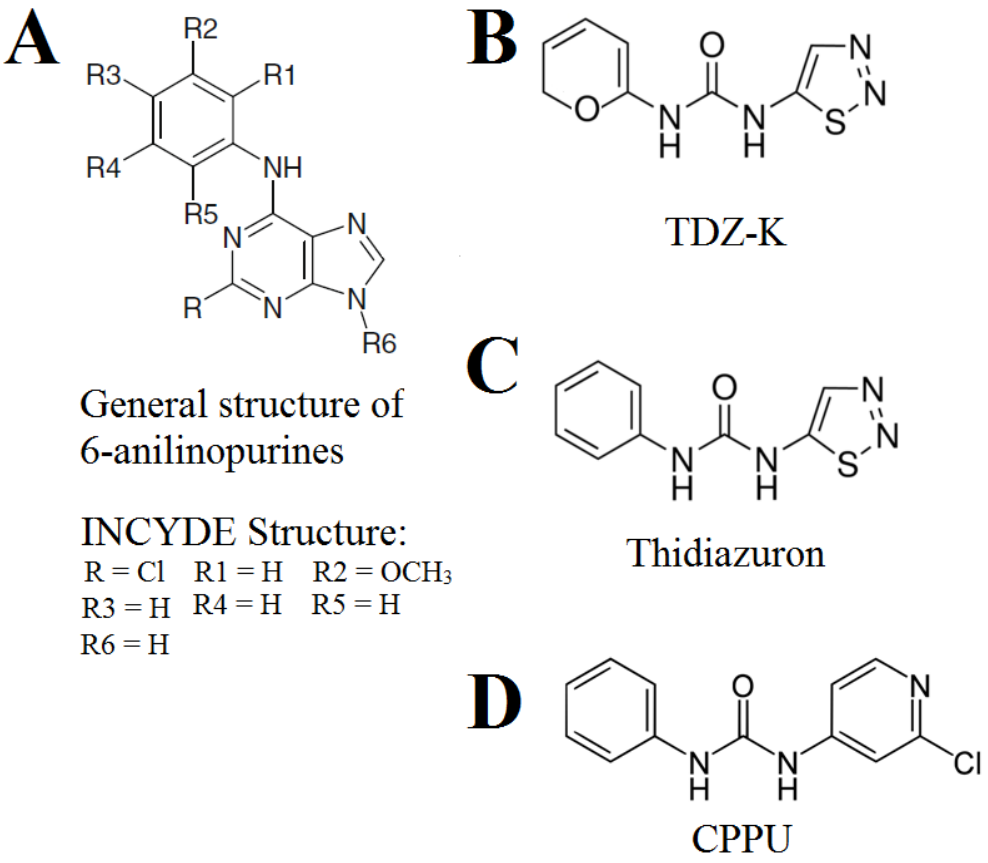

Figure 1.3 The chemical structures of plant growth regulators. A: general structure of 6-anilinopurines with the functional group substitutions of INCYDE for R to R6 described (from Zatloukal *et al*., 2008); B: TDZ-K; C: thidiazuron (TDZ); D: CPPU (forchlorfenuron).

1.3.2 TDZ-K

TDZ-K (*N*-furfuryl-*N*'-1,2,3-thiadiazol-5-yl-urea) is a diphenylurea (**Figure 1.3B**) derivative of thidiazuron (**Figure 1.3C**). TDZ-K does not inhibit CKX activity, but is known to not inhibit root growth (in contrast to TDZ) when applied at 100 nM with wheat and arabidopsis in root tests, and has a strong capacity to inhibit senescence in wheat and barley by blocking the degradation (and likely supporting the synthesis) of photosynthetic complexes within photosystem II (J. Nisler, personal communication, August 28, 2017; United States Patent US 2017/0280721 A1, 2017). Indeed, TDZ-K is known to retain chlorophyll content (at concentration ranges between 0.1 to 100 µM) better than TDZ or *t*Z in detached wheat leaf assays performed under dark and under dark/light conditions, and improve chlorophyll retention over BA in detached barley leaves under dark conditions (United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript).

In contrast to thidiazuron, TDZ-K showed reduced cytokinin activity, when tested on cytokinin-dependent *P. lunatus* callus tissue when applied at concentrations between 10 and 100 µM (Mok *et al*., 1982) and compared to thidiazuron, TDZ-K had a reduced ability to promote calcium ion-dependent ethylene production in mungbean hypocotyls when applied at 10 µM (Yip *et al*., 1986). Despite having an EC_{50} comparable with *t*Z in the *Amaranthus* bioassay (a classical cytokinin bioassay) and dose-dependent growth-promoting effects evident with tobacco callus bioassay (Nisler *et al*., unpublished manuscript), in contrast to cytokinins (such as BA), it does not have an inhibitory effect (or biphasic response) on tobacco callus growth at the highest concentrations tested (100 µM) (Nisler *et al*., unpublished manuscript).

In contrast to TDZ-K, thidiazuron has potent cytokinin activity and directly inhibits CKX activity (Chatfield and Armstrong, 1986; Hare and van Staden, 1994b; Nisler *et al*., 2016). Thidiazuron also shows strong cytokinin binding to cytokinin receptors (Mok *et al*., 1982; Yamada *et al*., 2001; Spíchal *et al*., 2004) and a strong capacity to inhibit senescence (Ferrante *et al*., 2002; Nisler *et al*., 2016). Thidiazuron is a known cotton defoliant (Arndt *et al*., 1976). The application of thidiazuron has a range of effects on growth, including increasing the number of shoots in seeds exposed to thidiazuron (Malik and Saxena, 1992; Singh *et al*., 2003; Zhihui *et al*., 2009), increasing fruit size (Piao *et al*., 2006) and fruiting rate (Wang *et al*., 2009), while excessive or long periods of application of thidiazuron have been observed to reduce root and shoot development (Ernst, 1994; Murthy *et al*., 1998).

1.3.3 CPPU

CPPU, or forchlorfenuron (*N*-(2-chloro-4-pyridyl)-*N*'-phenylurea), is a widely used PGR with a diphenylurea structure (**Figure 1.3D**). CPPU binds to CKX and inhibits its activity (Bilyeu *et al*., 2001; Nisler *et al*., 2016), and exhibits cytokinin activity in its capacity to bind to cytokinin receptors (Kopečný *et al.*, 2010). It can affect flower development including promoting earlier flowering in petunia under red light (Fukuda *et al*., 2016), and can alter floral morphology in *Torenia fournieri* Lind. (Nishijima and Shima, 2006). CPPU has a number of useful effects including increasing yield and fruit size in a number of plants including kiwifruit (Biasi *et al*., 1991), pear (Flaishman *et al*., 2006), apple (Stern *et al*., 2003), table grape (Ferrara *et al*., 2014), and Chinese white-flowered gourd (Yu *et al*., 1999), although some of these effects have come with less desirable side effects including abnormal growth (Tartarini *et al*., 1993). CPPU has also been shown to be able to induce shoot formation (Guo *et al*., 2005), delay senescence (Wang *et al*., 2009) and provide resistance against drought stress (Jianchang *et al*., 2003). CPPU has been applied at range of concentrations including 40 µM to fruits including apples and grapes (Stern *et al*., 2003; Ferrara *et al*., 2014), and up to 100 µM with cereals including Indica rice (Gashaw *et al*., 2014).

1.4 Aims and objectives

The initial aim of this project was to test the claims made for the novel PGR, INCYDE (Spichal *et al*., ACPD conference 2009, IPGSA conference 2010; Nisler *et al*., 2012, unpublished manuscript; Zatloukal *et al*., 2008; unpublished data, Palacký University). INCYDE has a strong capacity to inhibit cytokinin oxidase/dehydrogenase (CKX) (Zatloukal *et al*., 2008). Through its ability to prevent cytokinin degradation INCYDE is claimed to increase yield and to increase plant resistance to abiotic stressors (Reusche *et al*., 2013; Aremu *et al*., 2014; unpublished data, Palacký University). Subsequently, experiments were also designed to assess the claims made for TDZ-K and its ability to strongly inhibit senescence (United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript), and to determine whether a TDZ-K-induced inhibition of senescence translates into changes in growth and yield.

Based on knowledge of cytokinins and CKX in particular, and the proposed mode of action and reported effects of each PGR, the following were carried out:

Chapter 2: The efficacy of the PGRs INCYDE, TDZ-K and CPPU was assessed using the model plant RCBr, by monitoring growth, development, senescence and yield. This knowledge was used to develop a model to explain the effects of INCYDE and to inform the experimental design of field and small pot trials with wheat and barley cultivars with the three PGRs.

Chapter 3: Field trials were carried out with wheat and barley to evaluate the capacity of INCYDE, TDZ-K and CPPU to induce changes in growth, development, yield, grain composition and distribution, biomass, tillering and leaf senescence of wheat and barley.

Chapter 4: As cytokinins are reported to mitigate the impacts of stress on plants, INCYDE, TDZ-K and CPPU were used in trials under sub-optimal and stress conditions to test the hypothesis that a reduction in CKX (INCYDE and CPPU) or a delay in senescence (TDZ-K) would provide an enhancement of yield and/or mitigate from stress symptoms.

Chapter 5: An investigation into the effect of INCYDE, TDZ-K and CPPU on gene expression was carried out using samples from wheat and RCBr in order to determine the effect of each PGR on cytokinin homeostasis and, finally, to determine if the PGRs had an immediate impact on endogenous cytokinin levels in wheat and barley grains.

Chapter 2
Growth room experiments with rapid cycling *Brassica rapa*

2.1 Introduction

Rapid cycling *Brassica rapa* (Wisconsin Fast Plants®) abbreviated as RCBr, is a diploid, dicot model plant that was developed by the University of Wisconsin–Madison. Rapid cycling *B. rapa* is an ideal model plant for controlled growth room experiments: it has a rapid 40 to 50 day life cycle, a small, manageable adult size, large reproductive organs compared to other plant models such as arabidopsis (O'Keefe *et al*., 2011) and is closely-related to crop species including forage brassicas (*Brassica* spp.), turnip (B. *rapa*), rapeseed (*B. napus*), cauliflower, broccoli and cabbage (*B. oleracea* cultivars). It is also within the same family (Brassicaceae) of plants that were used in preliminary experiments with INCYDE (arabidopsis and *B. rapa*, canola; unpublished data, Palacký University).

2.1.1 INCYDE and TDZ-K

Novel compound INCYDE (2-chloro-6-(3-methoxyphenyl)aminopurine) was reported to have strong CKX-inhibiting properties, and to only bind to cytokinin receptors (Zatloukal *et al*., 2008). Preliminary experiments with INCYDE found that when applied as a spray at 10 and 50 µM INCYDE with winter rapeseed (*B. napus* L.), this resulted in increases in the branching, silique number and seed yield (unpublished data, Palacký University; personal communication, May 22, 2012). While with arabidopsis, four spray applications of 10 µM INCYDE early in development were required to enhance shoot growth, biomass, branching, seeds per silique and the overall seed yield (unpublished data, Palacký University; personal communication, May 22, 2012). As is typical of other PGRs and cytokinins, INCYDE has a biphasic response (inhibitory effect) at higher concentrations. Shoot and/or root growth was inhibited in *Rumex crispus* and *Bulbine natalensis* seedlings (where seeds germinated in water containing 10 µM INCYDE) and in arabidopsis plants following root drenching in solutions containing 10 to 100 nM INCYDE (in contrast to growth promotion at 1 nM INCYDE) (unpublished data, Palacký University). Collaborators at Palacký University suggested early application, before flowering (personal communication, May 22, 2012).

TDZ-K (*N*-furfuryl-*N*'-1,2,3-thiadiazol-5-yl-urea) is a diphenylurea that does not inhibit CKX (in contrast to INCYDE), is known to not inhibit wheat and arabidopsis root growth (when applied at 100 nM) and is able to inhibit wheat and barley leaf senescence in detached leaf assays (at concentration ranges between 0.1 to 100 µM) by blocking the degradation of photosynthetic complexes within photosystem II (J. Nisler, personal communication, August 28, 2017; United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript). However, there are no reports of TDZ-K inhibiting senescence in any *Brassica* species. Although TDZ-K shows cytokinin (or cytokinin-like) activity with its anti-senescence property and dose-dependent promotion of tobacco callus growth, in contrast to cytokinins, it shows no biphasic response at the concentration range tested (1 to 100 µM), in contrast to BA (Nisler *et al*., unpublished manuscript). The concentration ranges, along with the method of application (spraying) and timing of application (early in development) used in the Palacký University experiments with INCDYE and TDZ-K were replicated in this study.

2.1.2 Nitrogen

Plants require inorganic nitrogen in the forms of nitrate (NO_3^-), ammonium (NH_4^+) and urea for growth and development (Dechorgnat *et al*., 2010). Cytokinin concentrations and regulation of its activity are both affected by the availability of different nitrogen sources (Walch-Liu *et al*., 2000; Takei *et al*., 2004a; Kamada-Nobusada *et al*., 2013; Shtratnikova *et al*., 2015). The differential response of plants to nitrogen sources extends to nitrogen uptake, with low nitrate (< 1 mM KNO_3) inducing high affinity transport systems (HATS) (Krapp *et al*., 2014), while higher nitrate concentrations are taken up by low affinity transport systems (LATS) (Touraine and Glass, 1997; Glass and Kotur, 2013). Ammonium is taken up by ammonium-specific transporters (Engelsberger and Schulze, 2012). Understanding the influence of different nitrogen sources during the growth, yield and senescence of RCBr was important for this investigation in determining the efficacy of INCYDE and TDZ-K, and was also necessary for experimental optimisation and for determining whether there was a cytokinin-nitrogen interaction or nitrogen-dependent effect.

2.2 Materials and Methods

2.2.1 Rapid cycling *Brassica rapa* and growth room setup

Rapid cycling *B. rapa*, seeds were acquired from Wisconsin Fast Plants®. Fertiliser-free potting soil mix was placed into pots measuring 320 mm x 250 mm x 20 mm (width x length x depth). Rapid cycling *B. rapa* seeds were sown at a depth of 10 mm at an initial rate of eight to twelve plants per pot, and were provided with 24 h of florescent light (45 to 60 µmol $m^{-2}s^{-1}$), continuously over development, as recommended by the supplier. The growth room was 4 x 3 m in size, was continuously ventilated and maintained at 22°C. The different stages of development are described in **Figure 2.1A**. The point in time when each stage in development was first observed (with variation depending on the nitrogen source and experimental setup) was defined by days from the sowing date (0 d). These growth stages include the appearance of seedlings (2 to 4 d), true leaves (6 to 8 d), buds (8 to 15 d), flowers (13 to 20 d) and siliques (18 to 30 d) (**Figures 2.1A** and **B**). Trays were continuously provided with a water or a nutrient solution from the sowing date to the end of the RCBr life cycle (40 to 55 d). After seedling appearance, seedlings were thinned to three to four plants per pot, selecting out abnormal or under-developed seedlings. Trays were randomly arranged around the growth room, rearranged during development and regularly cleaned to reduce fungal growth and experimental contamination.

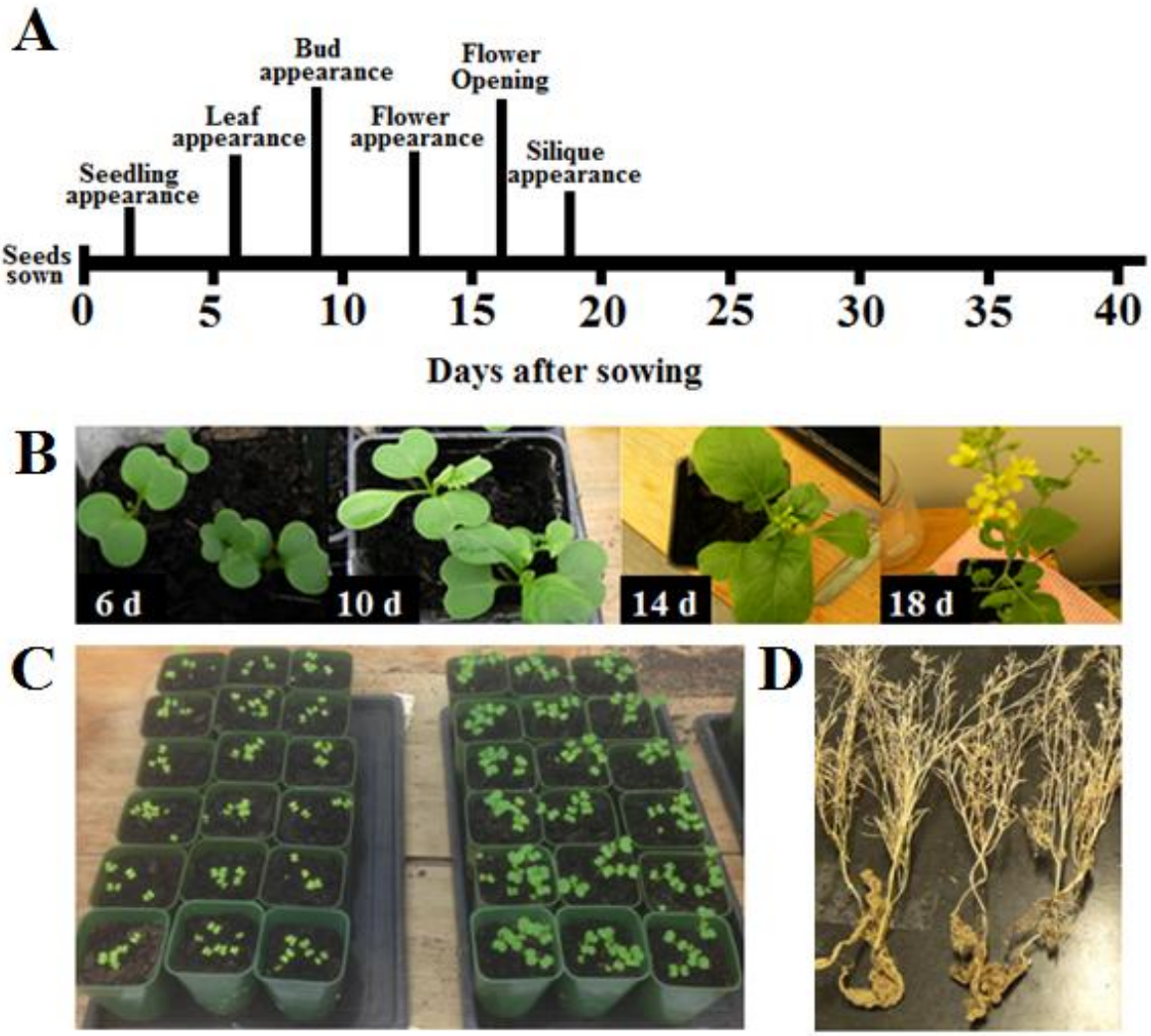

Figure 2.1 Development, experimental layout and harvesting of RCBr grown in a controlled growth room. A: Timeline of the progression of different stages in growth and development of RCBr. Time points refer to the earliest time these events were observed and days (d) are given from the sowing date; B: the appearance of RCBr seedlings and plants at 6, 10, 14 and 18 d; C: the layout of pots within treatment trays; and D: harvested air and oven dried plant samples prior to dry weight measurements.

2.2.2 Experimental design

The experiment was set up with a randomised complete block design, with three blocks (**Figure 2.2**). Each block was independently carried out at different dates in the growth room. Each block was made up of two or more treatment trays (the unit of replication), with individual trays representing a single treatment level replicate (**Figure 2.1C**). Treatment trays were randomly arranged within each block

and represented once in each block. Each tray contained 18 pots and each pot contained three to four plants.

2.2.2.1 Subsampling

For each replicate of a treatment level, the mean was calculated for a growth or yield trait (for example, individual seed mass) (**Figure 2.2**), by taking a random subsample of 30 plants from each tray within each of the three blocks. For chlorophyll analyses, a subsample of 9 whole plants was taken from each tray. The same number of samples were taken from each replicate for each treatment level. The overall mean for a treatment level was calculated using the three replicates and reported with the standard error in the results tables.

2.2.3 Plant growth regulators

INCYDE and TDZ-K were synthesised and provided by the Laboratory of Growth Regulators and the Institute of Experimental Botany (Palacký University). Each of these compounds were dissolved in dimethylsulfoxide (DMSO) (Scharlab) to produce 10 mM stock solutions which were stored at -20°C until use.

2.2.3.1 Working solutions

Working solutions were made by diluting stock solutions of INCYDE and TDZ-K to 25 µM in water poured into 500 mL spray bottles (McGregor's), and the solutions were mixed with surfactant Tween 20 (Sigma-Aldrich) at 0.1% (v/v). This concentration was within the experimental range described in preliminary experiments with both of these compounds (see sections **1.3.1**, **1.3.2** and **2.1.1**). Control solutions were produced by adding equivalent volumes of DMSO and of surfactant to water as used by the treatments. INCYDE treatments were applied four times before flowering at 8, 9, 10 and 12 d after sowing, and in a separate experiment once at 10 d after sowing. TDZ-K treatments were applied once before flowering at 10 d after sowing. These early (pre-anthesis) growth stages were recommended for each compound (as described in section **2.1.1**). Treatments were applied to whole plants, with focus on the foliar parts, until runoff, with 0.5 mL treatment applied per plant for each application.

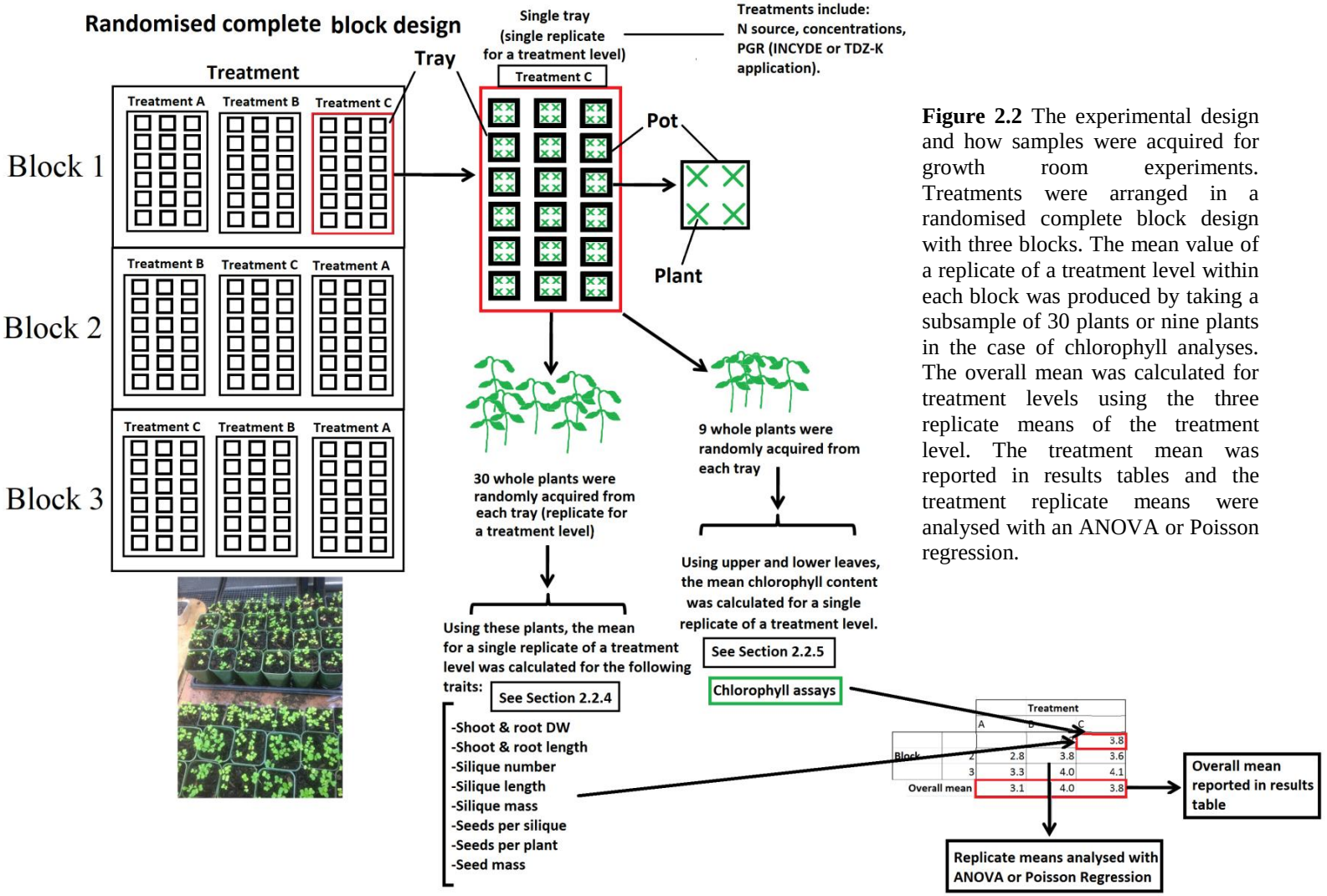

Figure 2.2 The experimental design and how samples were acquired for growth room experiments. Treatments were arranged in a randomised complete block design with three blocks. The mean value of a replicate of a treatment level within each block was produced by taking a subsample of 30 plants or nine plants in the case of chlorophyll analyses. The overall mean was calculated for treatment levels using the three replicate means of the treatment level. The treatment mean was reported in results tables and the treatment replicate means were analysed with an ANOVA or Poisson regression.

2.2.4 Maintenance and measurements

Measurements were made over each stage of development including the silique number and shoot height. The shoot height was defined as the length of the main stem from the top of the soil line to the highest point on the main stem, and the root was defined as everything below this soil line.

2.2.4.1 Pollination

Flowers were hand pollinated multiple times during flowering (from 13 to 25 d). Pollination was carried out at two to three day intervals to ensure that all flowers were pollinated.

2.2.4.2 Harvest, dry weight and yield determination

Plants were harvested at 45 to 50 days from the sowing date. Plant material was washed thoroughly and air dried for a week. Plant material was placed in an oven for 70 to 80°C for at least two hours or until the dry weight stabilised (**Figure 2.1D**). Measurements made post-harvest included shoot and root dry weights, shoot height, silique number, length and mass, the number of seeds per silique and individual seed mass. The number of seeds per plant was calculated by multiplying the average number of seeds per silique by the number of siliques per plant. The shoot was defined as all plant material (except the siliques) above the soil line. It was difficult to get accurate root DW measurements without losing a proportion of the root structure, root DW measurements were therefore given for the root crown.

2.2.5 Chlorophyll content determination

For chlorophyll analyses, leaves were categorised into older 'lower' leaves and younger 'upper' leaves. Leaf samples were excised from plants towards the end of leaf senescence (48 d). Leaf samples were immediately placed on ice after excision and then transported to a freezer to be stored at -20°C until analysis. Chlorophyll content was determined using a protocol described in Evans *et al*. (2012). For each treatment level, three independent replicates were acquired. For each replicate of a treatment level (each tray), nine plants were acquired. The leaf samples of these whole plants weighed between 0.1 and 70 mg (due to the large variation in leaf size between treatments). These leaf samples were placed in 200 µL dimethylformamide (DMF) (Sigma-Aldrich) to give samples that were no more than 0.35 mg FW/µL DMF. Samples were protected from UV by covering the samples in aluminium and samples were then incubated overnight at 4°C. Samples were centrifuged at 10 000 g for five min and then 2 µL of the chlorophyll extracts was measured using a NanoDrop® ND-1000 spectrophotometer

(Thermo Fisher Scientific) with software NanoDrop 1000 v 3.8.1. Chlorophyll content was calculated using calculations from Wellburn (1994) for chlorophyll a and b, where A_{664} and A_{647} represent the absorbance at these wavelengths:

$$\textbf{Chl A} = 11.65 \times A_{664} - 2.69 \times A_{647}$$
$$\textbf{Chl B} = 20.81 \times A_{647} - 4.53 \times A_{664}$$

A combined equation for total chlorophyll content was used for the protocol described in Evans *et al*. (2012). This equation allows the chlorophyll content to be calculated in mg/g FW.

$$\textbf{Chl}_{\textbf{total}} = 7.12\ A_{664} + 18.12\ A_{647}$$

Using the subsample of nine whole plants, the mean chlorophyll content was calculated for each treatment level replicate, and the overall mean for a treatment level was calculated from its three replicates and reported with the standard error in the results tables.

2.2.6 Statistical analyses

2.2.6.1 Nitrogen treatment experiments

In the nitrogen treatment experiments (section **2.3.1**), for each trait that was measured, an ANOVA, (significance level: 0.05, two sided) was carried out on the treatment replicate means using Minitab 17 (Minitab Inc.) (**Figure 2.3**). The ANOVA was partitioned for the following factors: the concentration of the nitrogen source (1, 5 and 15 mM), nitrogen source (KNO_3, NH_4Cl and NH_4NO_3), the block factor, as well as the interaction between the nitrogen source and concentration of the source. All factors were fixed. The *F*-values and *p*-values for the ANOVA for each trait are reported in the result tables.

To meet the assumption for the ANOVA of the residuals being normally distributed, a residual plot of the standardised residual was examined and reported with a Q-Q plot. The assumption of equality of the variances (homoscedasticity) was shown by plotting standardised residuals against predicted values to determine the distribution around 0. These residual plots were produced in Minitab 17 and reported in the Appendix described in each table. Where there were outliers in the data, which had an effect on the appearance of normality and homoscedasticity of the residual plots, these data points were checked, to determine if there was a calculation error and what effect this data point had on the outcome of the ANOVA. In each case, the data point was not a calculation error, nor did it

significantly influence the outcome of the ANOVA. Outliers were checked before every ANOVA test was carried out.

Following the ANOVA, where a factor (or interaction) had a *p*-value ≤ 0.05, a *post hoc* Tukey honest significant difference (HSD) test (confidence interval: 95%) was carried out to make multiple pairwise comparisons using adjusted *p*-values with XLSTAT 2016 (Addinsoft) and determine if there was a statistically significant difference between the means of treatment levels. Using the Tukey HSD, treatment levels were grouped according to significant differences and given a notation of a, b, c or d. If the interaction had a *p*-value ≤ 0.05, an interaction plot was also carried out and examined to determine the nature of this interaction effect (XLStat 2016).

For count data (such as the silique number), the count data was log-transformed (with $\log_{10}$) to satisfy the requirements of the parametric test. Residual plots were constructed to determine if these satisfied the assumptions of normality and homoscedasticitiy. The data was then analysed with ANOVA and a *post hoc* Tukey HSD tests if $p \leq 0.05$ for a factor. A Poisson regression was not used for the count data of the nitrogen experiments due to the need to use a Tukey HSD to make the appropriate pairwise comparisons.

2.2.6.2 INCYDE and TDZ-K treatment experiments

For experiments where INCYDE and TDZ-K were applied (sections **2.3.2** and **2.3.3**) the treatment replicate means were analysed with an ANOVA (significance level: 0.05, two sided) using Minitab 17 (Minitab Inc.) (**Figure 2.3**). The ANOVA looked at the treatment factor (main factor) and the block factor. All factors were fixed. The *F*-values and *p*-values were reported in each result table. Residual plots were carried out to meet the assumptions of the ANOVA (as described in section **2.2.6.1**). For the treatment factor, when the *p*-value was ≤ 0.05, a *post hoc* Tukey HSD test (Minitab 17) was carried out to determine if there was a statistically significant difference between the control and INCYDE treatment group, and these results were reported in the results table with an *. For count data, samples were analysed with a Poisson regression (log-link function, confidence interval: 95%, Two-sided) using Minitab 17, with the control set as the reference level, and the coefficients and *p*-values were examined to determine if there was a statistically significant difference between the control and INCYDE treatment.

2.2.6.3 Chlorophyll assays

For experiments where the chlorophyll content of RCBr leaves was analysed following INCYDE treatment at different nitrogen concentrations (section **2.3.4**), an ANOVA analysis (significance level: 0.05, two sided) was carried out (**Figure 2.3**) looking at the factors: the nitrogen (nitrate) concentration factor, the plant growth regulator (PGR) treatment factor, the block factor and an interaction between the nitrate concentration and PGR treatment (Minitab 17). All factors were fixed. Residual plots for ANOVA assumptions were carried out as described in section **2.2.6.1**.The *F*-values and *p*-values were reported in the results tables. Where $p \leq 0.05$ for any of the factors tested, a *post hoc* Tukey HSD was carried out in Minitab 17 to determine if there was a statistically significant difference between the control and INCYDE treatment at any nitrogen concentration, or between plants treated with INCYDE and control. Where there was statistically significant difference between the INCYDE group and the control group (e.g. a pairwise comparison of INCYDE vs. control), a ** was used to indicate this significant difference in the results table.

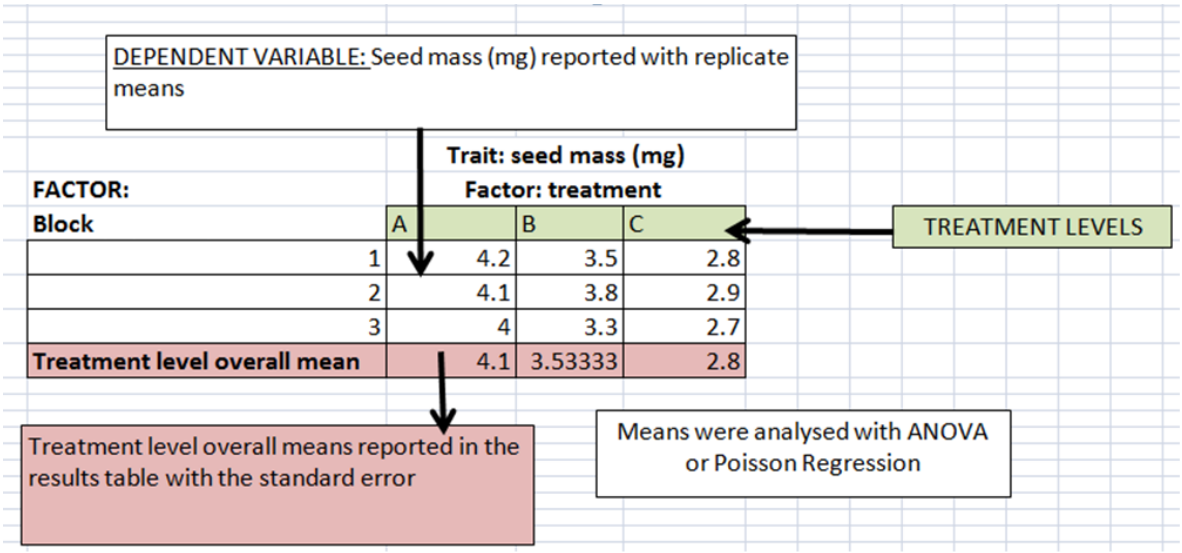

Figure 2.3 Means for each replicate of a treatment level used to perform ANOVA or a Poisson regression. Overall means are described in the tables of the Results section.

2.3 Results

2.3.1 Rapid cycling *Brassica rapa* grown under different nitrogen treatments

Rapid cycling *B. rapa* was grown under different nitrogen sources to establish the effect and suitability of each nitrogen source on the growth and development of RCBr (**Table 2.1**), and to determine the suitable nitrogen treatment for subsequent PGR experiments with INCYDE and TDZ-K. For the shoot DW, an ANOVA revealed (Appendix 2.1.1) statistically significant ($p \leq 0.05$) effect of the nitrogen concentration factor ($F_{2, 16} = 114.67$, $p < 0.001$), the nitrogen source ($F_{2, 16} = 42.18$, $p < 0.001$), and evidence of an interaction effect between the nitrogen source and concentration ($F_{4, 16} = 20.53$, $p < 0.001$). Examination of the interaction plot (Appendix 2.1.1) confirms that the lines are not parallel, that there is an interaction where the effect of each nitrogen source on growth is dependent on the concentration. The interaction is disordinal; with the NH_4Cl and KNO_3 treatments crossing over at two points, with the NH_4Cl treatment resulting in greater shoot DW at 5 mM compared to KNO_3, and less shoot DW for NH_4Cl when applied at 15 mM compared to 15 mM KNO_3. The interaction plot shows that the highest concentration of NH_4Cl resulted in a decrease in shoot DW, in contrast to KNO_3 and NH_4NO_3 treatments. The concentration of KNO_3 and NH_4NO_3 positively correlated with shoot DW with a statistically significant increase (*post hoc* Tukey HSD, confidence interval: 95%, Appendix 2.1.1) between 1, 5 and 15 mM for NH_4NO_3 and between 5 and 15 mM for KNO_3. The shoot DW was greatest at 5 mM when grown in NH_4Cl, which was a significant increase from plants grown at 1 mM, but at the highest concentration (15 mM) the plants grown under NH_4Cl had significantly reduced shoot DW compared to plants grown with nitrate-containing sources KNO_3 and NH_4NO_3.

For the silique number, ANOVA revealed (Appendix 2.1.2) a statistically significant ($p \leq 0.05$) effect of both the nitrogen concentration ($F_{2, 16} = 59.95$, $p < 0.001$), nitrogen source factors ($F_{2, 16} = 18.89$, $p < 0.001$) and an interaction effect ($F_{4, 16} = 3.85$, $p = 0.022$). An interaction plot (Appendix 2.1.2) shows that the KNO_3 and NH_4Cl lines are parallel at 1 and 5 mM, while NH_4NO_3 resulted in a larger silique number when applied at 5 mM. While the silique number decreased between 5 and 15 mM for both NH_4NO_3 and NH_4Cl, in contrast it increased for KNO_3. Pairwise comparisons reveal that the greatest number of siliques was measured in RCBr plants grown at 5 and 15 mM KNO_3 and NH_4NO_3 (*post hoc* Tukey HSD, confidence interval: 95%, Appendix 2.1.2) (**Table 2.1**), while for NH_4Cl, the silique number was greatest at 5 mM with a statistically significant decline at the highest concentration (15 mM) relative to when grown at 5 mM. For each of the nitrogen sources, there was a

significant increase in the silique number between 1 and 5 mM. The ANOVA revealed there was no statistically significant difference between the means of the silique length (Appendix 2.1.3) or the number of seeds per silique (Appendix 2.1.4) between plants grown in different nitrogen treatments (**Table 2.1**).

For seeds per plant, an ANOVA revealed (Appendix 2.1.5) that there was statistically significant effect of the nitrogen concentration ($F_{2, 16} = 175.21$, $p < 0.001$), nitrogen source ($F_{2, 16} = 77.34$, $p < 0.001$) and evidence of an interaction between the concentration and source ($F_{4, 16} = 20.19$, $p = 0.001$) (**Table 2.1**). An interaction plot (Appendix 2.1.5) confirmed an interaction, and a disordinal interaction with the NH_4NO_3 and KNO_3 lines crossing over between 5 and 15 mM. The number of seeds per plant decreased for NH_4NO_3 and NH_4Cl between 5 and 15 mM, in contrast to KNO_3, where it increased. The lines of the interaction plot indicate a similar pattern of the NH_4-containing sources NH_4NO_3 and NH_4Cl. Pairwise comparisons reveal that the greatest number of seeds per plant was evident at 5 and 15 mM in KNO_3 and NH_4NO_3 (*post hoc* Tukey HSD, confidence interval: 95%, Appendix 2.1.5), while at the highest concentration of NH_4Cl (15 mM), there was a significant decrease in the number of seeds per plant compared to 5 mM. Pairwise comparisons with Tukey HSD revealed a statistically significant increase in the number of seeds per plant as the concentration was increased from 1 to 5 mM for each of the different nitrogen sources used (NH_4NO_3, KNO_3 and NH_4Cl).

For individual seed mass, an ANOVA revealed (Appendix 2.1.6) a statistically significant effect of the nitrogen concentration ($F_{2, 16} = 22.75$, $p < 0.001$), N source ($F_{2, 16} = 6.04$, $p = 0.011$) and evidence of an interaction between the concentration and source ($F_{4, 16} = 11.86$, $p < 0.001$) (**Table 2.1**). An interaction plot (Appendix 2.1.6) revealed a disordinal interaction, with NH_4Cl, crossing over KNO_3 and NH_4NO_3 at 5 mM, with the largest individual seed mass at 5 mM with NH_4Cl. The lines of KNO_3 and NH_4NO_3 are nearly parallel, showing very little interaction effect when comparing these sources. Pairwise comparisons revealed that the greatest individual seed mass was evident at 1 mM and 5 mM KNO_3 and 5 mM NH_4Cl (*post hoc* Tukey HSD, confidence interval: 95%, Appendix 2.1.6). With KNO_3, there was a statistically significant decline in individual seed mass at 15 mM, compared to the lowest concentration (1 mM), while with NH_4Cl, a decline was evident at 15 mM compared to 5 mM. The concentrations of NH_4NO_3 did not result in any statistically significant difference in the individual seed mass.

Table 2.1 The effect of KNO_3, NH_4Cl and NH_4NO_3 at 1, 5 and 15 mM provided continuously over development on RCBr plants. The effect of each nitrogen source on shoot DW, silique number, silique length, seeds per silique, seeds per plant and seed mass is described. Plants were grown at a density of three plants per pot.

	KNO_3			NH_4Cl			NH_4NO_3		
Trait	1 mM	5 mM	15 mM	1 mM	5 mM	15 mM	1 mM	5 mM	15 mM
Shoot DW (mg)	68.4 ± 5.5 **cd**	108.4 ± 6.7 **cd**	396.6 ± 25.7 **a**	22.8 ± 2.9 **d**	133.9 ± 21.9 **bc**	106.7 ± 32.3 **cd**	58.6 ± 15.4 **cd**	237.7 ± 21.9 **b**	452.8 ± 34.0 **a**
Silique number	1.7 ± 0.6 **b**	7.6 ± 0.2 **a**	12.3 ± 1.1 **a**	1.3 ± 0.5 **b**	7.7 ± 0.6 **a**	2.8 ± 0.8 **b**	2.6 ± 0.3 **b**	15.5 ± 1.2 **a**	13.1 ± 1.7 **a**
Silique length (mm)	22.1 ± 1.0	28.6 ± 2.2	24.4 ± 2.7	20.7 ± 1.5	24.1 ± 1.6	25.6 ± 1.3	23.7 ± 1.4	21.0 ± 0.4	22.6 ± 1.0
Seeds per silique	5.2 ± 0.6	9.5 ± 1.3	7.7 ± 1.8	5.5 ± 1.0	6.3 ± 0.8	6.3 ± 0.5	6.8 ± 1.0	5.4 ± 0.1	7.1 ± 0.4
Seeds per plant	10.3 ± 1.9 **c**	68.8 ± 3.7 **ab**	127.2 ± 5.1 **a**	7.3 ± 1.3 **c**	47.3 ± 6.6 **b**	18.1 ± 7.7 **c**	17.8 ± 2.7 **c**	104.9 ± 7.4 **ab**	89.7 ± 3.6 **ab**
Individual seed mass (mg)	3.0 ± 0.1 **a**	2.7 ± 0.1 **ab**	2.4 ± 0.1 **bc**	2.5 ± 0.1 **bc**	3.1 ± 0.04 **a**	2.2 ± 0.1 **c**	2.7 ± 0.02 **ab**	2.3 ± 0.1 **bc**	2.3 ± 0.1 **bc**

	Summary of ANOVA results							
	F-values			*p*-values				
Trait	Conc. ($F_{2, 16}$)[e]	N source ($F_{2, 16}$)[e]	Interaction: Conc. * N source ($F_{4, 16}$)[e]	Conc.	N source	Interaction: Conc.* N source	Statistics	Analysis notes
Shoot DW (mg)	114.67	42.18	20.53	**< 0.001**	**< 0.001**	**< 0.001**	**Appendix 2.1.1**	ANOVA, Tukey HSD, interaction plot
Silique number	59.95	18.89	3.85	**< 0.001**	**< 0.001**	**0.022**	**Appendix 2.1.2**	log_{10} transformed, ANOVA, Tukey HSD, interaction plot
Silique length (mm)	1.76	1.91	2.58	0.203	0.180	0.077	**Appendix 2.1.3**	ANOVA
Seeds per silique	1.62	1.13	2.09	0.228	0.347	0.130	**Appendix 2.1.4**	log_{10} transformed, ANOVA, Tukey HSD, interaction plot
Seeds per plant	175.21	77.34	20.19	**< 0.001**	**< 0.001**	**0.001**	**Appendix 2.1.5**	log_{10} transformed, ANOVA, Tukey HSD, interaction plot
Individual seed mass (mg)	22.75	6.04	11.86	**< 0.001**	**0.011**	**< 0.001**	**Appendix 2.1.6**	ANOVA, Tukey HSD, interaction plot

abcd Indicates groupings of plants based on statistically significant differences established following an ANOVA (with $p \leq 0.05$ for treatment factor) with a *post hoc* Tukey HSD test (confidence interval of 95%).

[e] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

The values provided are overall means for three independent replicates (n = 3), presented with the standard error. For each treatment level, each replicate is made up of 30 randomly-selected plants taken from pots from a single tray, and the mean for each replicate generated from the subsample of 30 plants. Each replicate of a treatment level was represented once and randomly arranged within each of the three blocks to give a randomised complete block design. For more information on the experimental design and analyses see section **2.2.2**. Tests including ANOVA, *post hoc* Tukey HSD tests, log transformations and residual plots for the ANOVA assumptions are provided in **Appendices 2.1.1** to **2.1.6** for each trait. Details on harvesting and each trait are described in section **2.2.4**, and analyses are described in section **2.2.6.1**. *F*-values and *p*-values are provided for the factors: nitrogen concentration (1, 5 and 15 mM), nitrogen source (KNO_3, NH_4Cl, NH_4NO_3) and an interaction factor between the concentration and source.

2.3.2 INCYDE application with different nitrogen sources

The nitrogen experiments showed that KNO_3 was a suitable nitrogen source for testing the interactions with INCYDE as, in contrast to NH_4Cl, it did not inhibit components of growth or yield at higher concentrations. Experiments were carried out to determine the effect of four applications of INCYDE (as described with Palacký experiments) on the growth and yield of RCBr. The effect was investigated with a range of nitrate concentrations to determine if the nutrient source contributed to any observed effect. INCYDE was applied at 25 µM four times (8, 9, 10 and 12 d after sowing) before flowering with Tween 20 (0.1%) separately under 0.1, 1, 5 and 10 mM KNO_3 (**Tables 2.2** and **2.3**) or with fertiliser Tui Novatec Premium Fertiliser (at a rate of one pellet per plant) (**Table 2.4**). The only statistically significant difference measured was an increase in the number of seeds per plant following 25 µM INCYDE application when grown with 5 mM KNO_3 (**Table 2.3**) (Poisson regression: p = 0.02, confidence interval: 95%, Appendix 2.2.20). However, in plants grown with 5 mM KNO_3 and treated with INCYDE, ANOVA revealed that there was no other statistically significant differences in the shoot DW ($F_{1, 2}$ = 0.17, p = 0.723, Appendix 2.2.15), silique length ($F_{1, 2}$ = 10.42, p = 0.084, Appendix 2.2.17), silique mass ($F_{1, 2}$ = 2.08, p = 0.286, Appendix 2.2.18) or components of yield including silique number (Poisson regression: p = 0.379, Appendix 2.2.16), seeds per silique (Poisson regression: p = 0.897, Appendix 2.2.19) or seed mass ($F_{1, 2}$ = 1.53, p = 0.342, Appendix 2.2.21).

No statistically significant differences were found for any other trait following INCYDE treatment when plants were grown with 0.1 mM KNO_3, 1 mM KNO_3 (**Table 2.2**), 10 mM KNO_3 (**Table 2.3**) or fertiliser pellets (**Table 2.4**).

To determine if multiple applications were necessary for a yield enhancement under nitrate conditions (5 mM KNO_3) where yield enhancements were previously observed, plants were also provided with one application of 25 µM INCYDE (10 d after sowing), before flowering (**Table 2.5**). No statistically significant differences were measured for the silique number (Poisson regression: p = 0.98, Appendix 2.3.1), silique length ($F_{1, 2}$ = 0.15, p = 0.733, Appendix 2.3.2), seeds per silique (Poisson regression: p = 0.991, Appendix 2.3.3) or (in contrast to four applications) seeds per plant (Poisson regression: p = 0.815, Appendix 2.3.4).

Table 2.2 The effect of four applications of 25 µM INCYDE before anthesis on RCBr plants provided with 0.1 mM and 1 mM KNO_3. The effect on shoot DW, silique number, length, mass, seeds per silique, seeds per plant and seed mass is described. Plants were grown at a density of four plants per pot.

KNO_3 0.1 mM						
Trait	Control	INCYDE	Treatment *F*-values ($F_{1, 2}$)[a]	Treatment *p*-values	Statistics	Analysis notes
Shoot DW (mg)	8.8 ± 1.1	15.5 ± 5.5	1.09	0.407	**Appendix 2.2.1**	ANOVA
Silique number	0.6 ± 0.1	0.6 ± 0.1		1.0	**Appendix 2.2.2**	Poisson regression
Silique length (mm)	25.3 ± 0.3	26.2 ± 1.6	0.5	0.551	**Appendix 2.2.3**	ANOVA
Silique mass (mg)	20.0 ± 1.6	22.5 ± 2.3	12.14	0.073	**Appendix 2.2.4**	ANOVA
Seeds per silique	6.0 ± 0.5	5.4 ± 0.6		0.764	**Appendix 2.2.5**	Poisson regression
Seeds per plant	3.4 ± 0.6	3.3 ± 1.0		0.979	**Appendix 2.2.6**	Poisson regression
Individual seed mass (mg)	2.1 ± 0.2	2.2 ± 0.01	1.32	0.369	**Appendix 2.2.7**	ANOVA
KNO_3 1 mM						
Trait	Control	INCYDE	Treatment *F*-values ($F_{1, 2}$)[a]	Treatment *p*-values	Statistics	Analysis notes
Shoot DW (mg)	26.1 ± 10.5	25.1 ± 11.8	0.57	0.53	**Appendix 2.2.8**	ANOVA
Silique number	2.2 ± 0.5	1.9 ± 0.7		0.849	**Appendix 2.2.9**	Poisson regression
Silique length (mm)	34.0 ± 1.8	32.5 ± 0.6	0.65	0.504	**Appendix 2.2.10**	ANOVA
Silique mass (mg)	37.9 ± 1.1	35.4 ± 3.4	0.32	0.627	**Appendix 2.2.11**	ANOVA
Seeds per silique	9.3 ± 0.6	11.2 ± 0.1		0.457	**Appendix 2.2.12**	Poisson regression
Seeds per plant	19.1 ± 2.7	21.3 ± 7.6		0.552	**Appendix 2.2.13**	Poisson regression
Individual seed mass (mg)	2.3 ± 0.1	2.0 ± 0.2	3.57	0.2	**Appendix 2.2.14**	ANOVA

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

The values provided are overall means for three independent replicates (n = 3), presented with the standard error. For each treatment level, each replicate is made up of 30 randomly-selected plants taken from pots from a single tray, and the mean for each replicate generated from the subsample of 30 plants. Each replicate of a treatment level was represented once and randomly arranged within each of the three blocks to give a randomised complete block design. For more information on the experimental design and analyses see section **2.2.2**. Plants were treated four times at 8, 9, 10 and 12 d after sowing. Tests including ANOVA, Poisson regression and residual plots for the ANOVA assumptions are provided in **Appendices 2.2.1** to **2.2.14** for each trait. Details on harvesting and each trait are described in section **2.2.4**, and analyses are described in section **2.2.6.2**. *F*-values and *p*-values are provided for non-count data (shoot DW, silique length, silique mass and individual seed mass) for the treatment factor (INCYDE and Control). For count data (silique number, seeds per silique and seeds per plant), a *p*-value is provided for the treatment factor of a Poisson regression.

Table 2.3 The effect of four applications of 25 µM INCYDE before anthesis on RCBr plants provided with 5 mM and 10 mM KNO_3. The effect on shoot DW, silique number, length, mass, seeds per silique, seeds per plant and seed mass is described. Plants were grown at a density of four plants per pot.

KNO_3 5 mM						
Trait	Control	INCYDE	Treatment *F*-values ($F_{1, 2}$)[a]	Treatment *p*-values	Statistics	Analysis notes
Shoot DW (mg)	183.9 ± 96.9	186.2 ± 91.9	0.17	0.723	**Appendix 2.2.15**	ANOVA
Silique number	5.2 ± 1.4	7.0 ± 2.3		0.379	**Appendix 2.2.16**	Poisson regression
Silique length (mm)	37.2 ± 1.6	39.7 ± 0.8	10.42	0.084	**Appendix 2.2.17**	ANOVA
Silique mass (mg)	50.8 ± 2.1	46.6 ± 1.2	2.08	0.286	**Appendix 2.2.18**	ANOVA
Seeds per silique	11.9 ± 1.2	12.3 ± 2.6		0.897	**Appendix 2.2.19**	Poisson regression
Seeds per plant	54.3 ± 7.0	**74.5 ± 4.8***		**0.002**	**Appendix 2.2.20**	Poisson regression
Individual seed mass (mg)	1.6 ± 0.8	2.3 ± 0.4	1.53	0.342	**Appendix 2.2.21**	ANOVA
KNO_3 10 mM						
Trait	Control	INCYDE	Treatment *F*-values ($F_{1, 2}$)[a]	Treatment *p*-values	Statistics	Analysis notes
Shoot DW (mg)	222.2 ± 56.5	247.5 ± 50.2	5.15	0.151	**Appendix 2.2.22**	ANOVA
Silique number	6.4 ± 0.8	5.7 ± 0.7		0.707	**Appendix 2.2.23**	Poisson regression
Silique length (mm)	37.4 ± 0.8	35.7 ± 1.8	0.48	0.559	**Appendix 2.2.24**	ANOVA
Silique mass (mg)	38.8 ± 3.5	42.7 ± 4.4	0.4	0.593	**Appendix 2.2.25**	ANOVA
Seeds per silique	12.9 ± 1.5	12.4 ± 2.7		0.887	**Appendix 2.2.26**	Poisson regression
Seeds per plant	78.3 ± 2.2	73.1 ± 1.0		0.458	**Appendix 2.2.27**	Poisson regression
Individual seed mass (mg)	2.0 ± 0.3	2.1 ± 0.3	0.09	0.798	**Appendix 2.2.28**	ANOVA

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

* Indicates a statistically significant difference for the treatment compared to the control with Poisson regression (95% confidence interval).

The values provided are overall means for three independent replicates (n = 3), presented with the standard error. For each treatment level, each replicate is made up of 30 randomly-selected plants taken from pots from a single tray, and the mean for each replicate generated from the subsample of 30 plants. Each replicate of a treatment level was represented once and randomly arranged within each of the three blocks to give a randomised complete block design. Overall means are described with the standard error. For more information on the experimental design and analyses see section **2.2.2**. Plants were treated four times at 8, 9, 10 and 12 d after sowing. Tests including ANOVA, Poisson regression and residual plots for the ANOVA assumptions are provided in **Appendices 2.2.15** to **2.2.28** for each trait. Details on harvesting and each trait are described in section **2.2.4**, and analyses are described in section **2.2.6.2**. *F*-values and *p*-values are provided for non-count data (shoot DW, silique length, silique mass and individual seed mass) for the treatment factor (INCYDE and Control). For count data (silique number, seeds per silique and seeds per plant), a *p*-value is provided for the treatment factor of a Poisson regression.

Table 2.4 The effect of four applications of 25 µM INCYDE before anthesis on RCBr plants provided with fertiliser pellets (1 per plant). The effect on shoot DW, silique number, length, mass, seeds per silique, seeds per plant and seed mass is described. Plants were grown at a density of four plants per pot.

Fertiliser pellets						
Trait	Control	INCYDE	Treatment *F*-values ($F_{1, 2}$)[a]	Treatment *p*-values	Statistics	Analysis notes
Shoot DW (mg)	141.3 ± 101.2	157.0 ± 117.7	0.9	0.443	**Appendix 2.2.29**	ANOVA
Silique number	5.1 ± 1.6	5.5 ± 2.2		0.855	**Appendix 2.2.30**	Poisson regression
Silique length (mm)	35.6 ± 4.2	33.6 ± 2.2	0.79	0.468	**Appendix 2.2.31**	ANOVA
Silique mass (mg)	35.0 ± 4.2	32.4 ± 1.6	0.63	0.509	**Appendix 2.2.32**	ANOVA
Seeds per silique	12.8 ± 1.6	11.9 ± 1.3		0.77	**Appendix 2.2.33**	Poisson regression
Seeds per plant	59.1 ± 9.5	58.1 ± 18.2		0.871	**Appendix 2.2.34**	Poisson regression
Individual seed mass (mg)	1.7 ± 0.3	1.7 ± 0.1	< 0.01	0.995	**Appendix 2.2.35**	ANOVA

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).
The values provided are overall means for three independent replicates (n = 3), presented with the standard error. For each treatment level, each replicate is made up of 30 randomly-selected plants taken from pots from a single tray, and the mean for each replicate generated from the subsample of 30 plants. Each replicate of a treatment level was represented once and randomly arranged within each of the three blocks to give a randomised complete block design. Overall means are described with the standard error. For more information on the experimental design and analyses see section **2.2.2**. Plants were treated four times at 8, 9, 10 and 12 d after sowing. Tests including ANOVA, Poisson regression and residual plots for the ANOVA assumptions are provided in **Appendices 2.2.29** to **2.2.35** for each trait. Details on harvesting and each trait are described in section **2.2.4**, and analyses are described in section **2.2.6.2**. *F*-values and *p*-values are provided for non-count data (shoot DW, silique length, silique mass and individual seed mass) for the treatment factor (INCYDE and Control). For count data (silique number, seeds per silique and seeds per plant), a *p*-value is provided for the treatment factor of a Poisson regression.

Table 2.5 The effect of a single application of 25 µM INCYDE before anthesis on RCBr plants provided with 5 mM of KNO_3. The effect on silique number, silique length, seeds per silique and seeds per plant is described. Plants were grown at a density of four plants per pot.

Trait	Control	INCYDE	Treatment *F*-values ($F_{1, 2}$)[a]	Treatment *p*-values	Statistics	Analysis notes
Silique number	5.7 ± 1.2	5.7 ± 1.0		0.98	**Appendix 2.3.1**	Poisson regression
Silique length (mm)	22.7 ± 0.8	21.8 ± 1.6	0.15	0.733	**Appendix 2.3.2**	ANOVA
Seeds per silique	5.7 ± 0.8	5.6 ± 0.3		0.991	**Appendix 2.3.3**	Poisson regression
Seeds per plant	31.2 ± 3.8	32.3 ± 3.3		0.815	**Appendix 2.3.4**	Poisson regression

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).
The values provided are overall means for three independent replicates (n = 3), presented with the standard error. For each treatment level, each replicate is made up of 30 randomly-selected plants taken from pots from a single tray, and the mean for each replicate generated from the subsample of 30 plants. Each replicate of a treatment level was represented once and randomly arranged within each of the three blocks to give a randomised complete block design. Overall means are described with the standard error. For more information on the experimental design and analyses see section **2.2.2**. Plants were treated once at 10 d after sowing. Tests including ANOVA, Poisson regression and residual plots for the ANOVA assumptions are provided in **Appendices 2.3.1** to **2.3.4** for each trait. Details on harvesting and each trait are described in section **2.2.4**, and analyses are described in section **2.2.6.1**. *F*-values and *p*-values are provided for non-count data (silique length) for the treatment factor (INCYDE and Control). For count data (silique number, seeds per silique and seeds per plant), a *p*-value is provided for the treatment factor of a Poisson regression.

2.3.3 Single applications of TDZ-K grown with fertiliser

To determine if TDZ-K could alter yield or growth, TDZ-K was applied once at 25 µM before flowering (10 d after sowing) with Tween 20 (0.1%) and supplied with fertiliser (at a rate of one pellet per pot) (**Table 2.6**). An ANOVA revealed there was no statistically significant difference between the TDZ-K and control group in the growth characteristics including the shoot height ($F_{1, 2}$ = 16.4, p = 0.056, Appendix 2.4.1), root length ($F_{1, 2}$ = 0.69, p = 0.495, Appendix 2.4.2), shoot DW ($F_{1, 2}$ = 10.68, p = 0.082, Appendix 2.4.3) and root DW ($F_{1, 2}$ = 6.99, p = 0.118, Appendix 2.4.4). Likewise, there was no significant difference in the yield characteristics between the treatment and control including the silique number (Poisson regression: p = 0.678, Appendix 2.4.5), silique mass ($F_{1, 2}$ = 1.5, p = 0.553, Appendix 2.4.6), seeds per silique (Poisson regression: p = 0.896, Appendix 2.4.7) and seeds per plant (Poisson regression: p = 0.24, Appendix 2.4.8).

2.3.4 The effect of INCYDE application on chlorophyll content

To determine the effect of INCYDE on senescence, the chlorophyll content of RCBr plants was measured at 48 d after sowing following four applications of 25 µM INCYDE (8, 9, 10 and 12 d after sowing applied with 0.1% Tween 20) under 0.1, 1, 5 and 10 mM KNO_3 nitrogen conditions (**Table 2.7**). For the upper leaves, ANOVA analysis revealed that there was no difference in the content of chlorophyll in INCYDE-treated plants compared to control plants ($F_{1, 10}$ = 0.58, p = 0.464, Appendix 2.5.1) or any interaction effect (or significant difference at any given nitrogen concentration) ($F_{2, 10}$ = 0.17, p = 0.849). The only significant difference in the content of chlorophyll in the upper leaves at this point in development was at different concentrations of nitrogen ($F_{2, 10}$ = 11.94, p = 0.002), where plants grown in 10 mM KNO_3 had significantly greater chlorophyll retention compared to plants grown in 1 or 5 mM KNO_3 (*post hoc* Tukey HSD, confidence interval: 95%, Appendix 2.5.1).

For the lower leaves there was a more rapid decrease in the content of chlorophyll in plants treated with INCYDE compared to control plants ($F_{1, 10}$ = 4.81, p = 0.046, pairwise comparison of control vs. INCYDE, *post hoc* Tukey HSD, confidence interval: 95%, Appendix 2.5.2). There was no interaction effect ($F_{2, 10}$ = 2.53, p = 0.099) or difference between INCYDE or control for a specific concentration (for example, from a pairwise comparison of 1 mM KNO_3 control vs. 1 mM KNO_3 INCYDE) (*post hoc* Tukey HSD, confidence interval: 95%, Appendix 2.5.2). In the lower leaves there was also a significant difference in the content of chlorophyll at different nitrogen concentrations ($F_{2, 10}$ = 10.17, p = 0.001), with leaves from plants grown in 10 mM KNO_3 having greater retention of chlorophyll compared to plants grown at either 1 or 0.1 mM KNO_3 (*post hoc* Tukey HSD, confidence interval:

95%, Appendix 2.5.2). Preliminary chlorophyll assays were carried out following a single 25 µM TDZ-K application, however, these experiments were discontinued due to a lack of any effect in early replicates.

Table 2.6 The effect a single application of 25 µM TDZ-K before anthesis on RCBr plants provided with fertiliser pellets (1 per plant). The effect on shoot height, root length, shoot DW, root DW, silique number, silique mass, seeds per silique and seeds per plant is described. Plants were grown at a density of four plants per pot.

Trait	Control	TDZ-K	Treatment *F*-values ($F_{1, 2}$)[a]	Treatment *p*-values	Statistics	Analysis notes
Shoot height (mm)	263.9 ± 1.6	235.5 ± 5.5	16.4	0.056	**Appendix 2.4.1**	ANOVA
Root length (mm)	41.1 ± 1.1	39.4 ± 1.2	0.69	0.495	**Appendix 2.4.2**	ANOVA
Shoot DW (mg)	72.1 ± 1.7	66.4 ± 2.1	10.68	0.082	**Appendix 2.4.3**	ANOVA
Root DW (mg)	12.1 ± 0.1	11.5 ± 0.1	6.99	0.118	**Appendix 2.4.4**	ANOVA
Silique number	5.8 ± 0.5	5.0 ± 0.4		0.678	**Appendix 2.4.5**	Poisson regression
Silique mass (mg)	35.7 ± 2.0	34.1 ± 1.8	1.5	0.553	**Appendix 2.4.6**	ANOVA
Seeds per silique	11.5 ± 0.8	11.9 ± 0.6		0.896	**Appendix 2.4.7**	Poisson regression
Seeds per plant	64.6 ± 7.7	57.1 ± 5.7		0.240	**Appendix 2.4.8**	Poisson regression

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

The values provided are overall means for three independent replicates (n = 3), presented with the standard error. For each treatment level, each replicate is made up of 30 randomly-selected plants taken from pots from a single tray, and the mean for each replicate generated from the subsample of 30 plants. Each replicate of a treatment level was represented once and randomly arranged within each of the three blocks to give a randomised complete block design. Overall means are described with the standard error. For more information on the experimental design and analyses see section **2.2.2**. Plants were treated once at 10 d after sowing. Tests including ANOVA, Poisson regression and residual plots for the ANOVA assumptions are provided in **Appendices 2.4.1** to **2.4.8** for each trait. Details on harvesting and each trait are described in section **2.2.4**, and analyses are described in section **2.2.6.2**. *F*-values and *p*-values are provided for non-count data (shoot height, root length, shoot DW, root DW and silique mass) for the treatment factor (INCYDE and Control). For count data (silique number, seeds per silique and seeds per plant), a *p*-value is provided for the treatment factor of a Poisson regression.

Table 2.7 The effect of four applications of 25 µM INCYDE before anthesis on RCBr plants provided with 0.1, 1, 5 and 10 mM of KNO_3 on chlorophyll content of upper and lower leaves at 48 d after sowing. Plants were grown at density of four plants per pot.

	Chlorophyll content (mg/g) for each treatment							
	KNO_3 0.1 mM		KNO_3 1 mM		KNO_3 5 mM		KNO_3 10 mM	
Leaf sample	Control	INCYDE	Control	INCYDE	Control	INCYDE	Control	INCYDE
Chlorophyll content upper leaf (mg/g)	No leaf[a]	No leaf[a]	0.7 ± 0.2	0.4 ± 0.1	1.1 ± 0.4	0.8 ± 0.2	2.0 ± 0.2	2.0 ± 0.5
Chlorophyll content lower leaf (mg/g)**	0.5 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	0.6 ± 0.1	1.6 ± 0.3	0.7 ± 0.3	2.2 ± 0.2	1.5 ± 0.3

	Summary of ANOVA results							
	F-values			*p*-values				
Leaf sample	N Conc. ($F_{2, 10}$)[b] ($F_{3, 14}$)[b]	PGR Treatment ($F_{1, 10}$)[b] ($F_{1, 14}$)[b]	Interaction: N conc. * PGR ($F_{2, 10}$)[b] ($F_{3, 14}$)[b]	N Conc.	PGR Treatment	Interaction: N conc. * PGR	Statistics	Analysis notes
Chlorophyll content upper leaf (mg/g)	11.94	0.58	0.17	**0.002**	0.464	0.849	**Appendix 2.5.1**	ANOVA, Tukey HSD
Chlorophyll content lower leaf (mg/g)	10.17	4.81	2.53	**0.001**	**0.046**	0.099	**Appendix 2.5.2**	ANOVA, Tukey HSD

[a] No upper leaves were present in plants grown in nitrogen-limited 0.1 mM KNO_3 conditions.
[b] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).
** Indicates a statistically significant difference (post hoc Tukey HSD, confidence interval: 95%) of the treatment compared to the control in a leaf sample.
The values provided are overall means for three independent replicates (n = 3), presented with the standard error. For each treatment level, each replicate is made up of nine randomly-selected plants taken from pots from a single tray. Upper and lower leaf samples are acquired from each plant. Each replicate of a treatment level was represented once and randomly arranged within each of the three blocks to give a randomised complete block design. For more information on the experimental design and analyses see section **2.2.2**. Plants were treated four times at 8, 9, 10 and 12 d after sowing. Tests including ANOVA, *post hoc* Tukey HSD tests and residual plots for the ANOVA assumptions are provided in **Appendices 2.5.1** and **2.5.2** for each trait. Details on chlorophyll analyses are described in section **2.2.5** and based on Evans *et al*., 2012, and analyses are described in section **2.2.6.3**. *F*-values and *p*-values are provided for the factors: nitrogen (nitrate) concentration (0.1, 1, 5 and 10 mM), treatment (control and INCYDE) and an interaction factor between the nitrogen concentration and treatment.

2.4 Discussion

2.4.1 Nitrogen sources and rapid cycling *Brassica rapa*

Growth room experiments with RCBr provided with different nitrogen forms (KNO_3, NH_4Cl, NH_4NO_3) at a range of concentrations (1, 5, 15 mM) were carried out to establish suitable nitrogen sources, to determine the contributing role of each nitrogen source to RCBr growth and yield, and to optimise growth conditions before PGR application experiments were carried out.

Nitrate is considered an important source of nitrogen, with evidence suggesting that using ammonium as an alternative source results in a reduction in growth and yield in arabidopsis (Walch-Liu *et al*., 2000; Shtratnikova and Kulaeva, 2008; Hachiya *et al*., 2012; Shtratnikova *et al*., 2015). An inhibition in growth and yield between NH_4Cl and nitrate-containing sources is most evident at the highest concentration tested (15 mM) (**Table 2.1**). The reduction in components of yield (silique number, seeds per plant and seed mass) when increasing the concentration of NH_4Cl from 5 to 15 indicates the toxicity of ammonium at higher concentrations when nitrogen is supplied as ammonium as a sole source. An ammonium concentration of 10 mM would be considered among the higher levels found in agricultural soil (Britto *et al*., 2001).

Several explanations and mechanisms have been proposed to explain the effects from ammonium toxicity (Britto and Kronzucker, 2002; Hachiya *et al*., 2012). The depletion of inorganic cations and organic acids in plant tissues was one cause that has been considered, although experiments where ammonium toxicity was alleviated by nitrate were not accompanied by a reduction in the depletion of inorganic cations and organic acids, or a reduction in the accumulation of ammonium (Hachiya *et al*., 2012). Additionally, is also possible that toxicity is due in part to the presence of chloride in NH_4Cl which can contribute towards plant toxicity (Tavakkoli *et al*., 2010).

At the highest concentrations tested where the effects of ammonium toxicity were observed, an enhancement of RCBr growth and yield was evident when both nitrate and ammonium were provided (NH_4NO_3) compared to when ammonium was provided as a sole source (NH_4Cl) (**Table 2.1**). This aligns with the other studies that show that the presence or addition of nitrate alleviates or prevents the symptoms of ammonium toxicity (Britto and Kronzucker, 2002; Garnica *et al*., 2009; Hachiya *et al*., 2012). Even though there are a limited number of species that can utilise ammonium as the primary

nitrogen source (Kronzucker *et al*., 1997), most plants require a mixture of nitrate and ammonium for optimal growth (Cao *et al*., 1993; Shtratnikova *et al*., 2015).

At lower concentrations (5 mM) the presence of both nitrate and ammonium (NH_4NO_3) provided a growth (shoot DW) advantage over plants supplied with KNO_3 as a sole source (**Table 2.1**), indicating that the greater overall content of N in NH_4KNO_3, provides a clear growth advantage in growth traits, but not a statistically significant enhancement of yield traits. Seed mass showed the opposite pattern, with plants grown in 5 mM NH_4Cl having a significantly greater mass compared to when provided with NH_4NO_3. These results collectively indicated the complex interaction between nitrate and ammonium metabolism and uptake, where the concentration of either is known to be able to enhance or reduce the uptake of the other (Criddle *et al*., 1988; Kronzucker *et al*., 1999; Garnica *et al*., 2009).

These experiments provided evidence for the suitability of nitrate as the primary nitrogen source for experiments with PGRs. This evidence includes the toxicity problems with using ammonium as a sole source and the previously described complexities introduced by using a mixed nitrogen source (NH_4NO_3) (**Table 2.1**). Given these observations, KNO_3 was selected as a suitable nitrogen source for investigating the efficacy of the PGRs on RCBr growth and yield. Fertiliser pellets were also used to determine the effects of a more conventional nutrient source, which could provide findings with relevant implications for pot trials.

2.4.2 Effect of INCYDE on growth and yield

The yield enhancement (in seeds per plant) when plants were grown in 5 mM KNO_3 and treated with four applications of 25 µM INCYDE (**Table 2.3**), is in agreement with increases reported for yield for arabidopsis following multiple applications of INCYDE (unpublished, Palacký University) and is an observation consistent with the purported capacity of INCYDE to inhibit CKX and enhance cytokinin content (Zatloukal *et al*., 2008). In the previous unpublished experiments, INCYDE affected a broad range of characteristics including the seed yield, silique number, seeds per silique, branching, shoot length, biomass, and the size of various components of flowers. In contrast, the effect of INCYDE on RCBr was very narrow and specific, it only affected the overall seed yield, and this effect was specific only to the nitrogen source provided (5 mM KNO_3) and required multiple applications prior to flowering, as single applications failed to reproduce this yield enhancement (**Table 2.5**).

There are several possible reasons for this significant disparity in effect; the surfactant (0.01% Tween 20) used in this study was different from that used previously with *B. napus* and arabidopsis where Silwet 806 was applied at 0.01%. Non-ionic surfactants facilitate delivery, adsorption, retention and uptake of PGRs (O'Sullivan *et al*., 1981), and the choice of surfactant, and the concentration used can significantly affect uptake (Stowe, 1960; Parr and Norman, 1964). Silwet belongs to a group of "super-spreaders" with low surface tension, low coefficient of friction and low interfacial tension, which enables rapid wetting, good foliar coverage and adhesion (Zhang *et al*., 2006). Therefore this could provide a partial explanation of this disparity in effect.

Another reason for this difference might be that previous experiments were carried out with winter rapeseed (*Brassica napus* L.), a *Brassica* sp. which would have grown to a much larger size than RCBr and is more suitable to grow in agricultural settings. Physiological, genetic or morphological differences between these species could help explain these disparities in effect. The winter rapeseed experiment was also carried out with some field conditions (although little detail of what field conditions were used was not provided with this), but this would involve natural sunlight (in comparison to growth room light) and introduce other environmental factors to the experiment. Other possible reasons for this disparity might include the lower density of plants per pot with Palacký University (only one plant appeared to be grown in each pot) as well as other growth conditions and methods of application. A number of these details on these aspects were not provided by Palacký University.

The effect of INCYDE was specific to the nitrogen source present. INCYDE inhibits CKX activity (Zatloukal *et al*., 2008), which alters the content of endogenous cytokinin. Given this, and the fact that cytokinin and nitrogen are known to interact (Takei *et al*., 2004a; Kamada-Nobusada *et al*., 2013; Shtratnikova *et al*., 2015), indicates that this nitrogen-specific effect may be due to the interaction of cytokinin and nitrogen.

The timing of INCYDE treatment was another factor to consider. Significant increases were not measured when INCYDE was targeted at flowering or later (data not shown), but only occurred when plants were targeted before flowering (**Table 2.3**). This suggested that earlier stages were developmentally critical for altering yield. This aligns with experiments with arabidopsis where yield enhancements were observed when INCYDE was targeted at growth stages one to five (using growth stages described in Boyes *et al*., (2001)), that is, before flowering (unpublished, Palacký University). The development of the reproductive shoot apical meristem over this period might help explain this

growth stage specific yield enhancement. This would align with Bartrina *et al*. (2011), where arabidopsis mutants with decreased *AtCKX* expression in the reproductive shoot apical meristems resulted in an increase in cytokinin, which consequentially led to an increase in the number of flowers and seeds. The specific organs that were targeted is also another important factor.

2.4.3 Modelling the effect of INCYDE

It was evident that four applications of INCYDE with 5 mM KNO_3 were required to enhance yield (**Table 2.3**), while single applications (**Table 2.5**), or applications with different nitrogen concentrations or sources had no effect on seed yield (**Tables 2.2**, **2.3** and **2.4**). This led to the development of a feedback model to explain the response of RCBr to INCYDE (**Figure 2.4**).

The model proposes that following INCYDE application, CKX activity is inhibited (Zatloukal *et al*., 2008) and endogenous cytokinin levels increased. Subsequently, the increase in cytokinin activates a feedback response involving the upregulation of cytokinin oxidase/dehydrogenase (*CKX*) expression and/or activity. There is evidence to support a cytokinin degradation feedback response following increases in cytokinin (Chatfield and Armstrong, 1986; Kamínek and Armstrong, 1990; Motyka and Kamínek, 1990; Motyka *et al*., 1996; 2003; Brugière *et al*., 2003; Blagoeva *et al*., 2004; Hirose *et al*., 2008; Vyroubalová *et al*., 2009; Jameson and Song, 2016). An alternative or additional response to the increased endogenous cytokinin might include a reduction in isopentenyltransferase (*IPT*) expression and/or activity. There is also evidence for a downregulation of some *IPT*s following BA treatment in Chinese cabbage (Liu *et al*., 2013) and arabidopsis (Miyawaki *et al*., 2004).

These feedback responses could counter this INCYDE-induced increase or even result in a temporary decrease in the concentration of endogenous cytokinins, which might in turn be subsequently stabilised by a combination of upregulation of *IPT*, downregulation of *CKX* and/or transport of cytokinin from other sources. This final step might help explain the lack of an increase in yield when only one application was made (**Table 2.7**), as one application might have failed to overcome this feedback response during a critical period of development involved in determining yield. Four applications in contrast, might have been sufficient to overwhelm the feedback by ensuring endogenous cytokinin levels were maintained at sufficiently high levels over this critical development period.

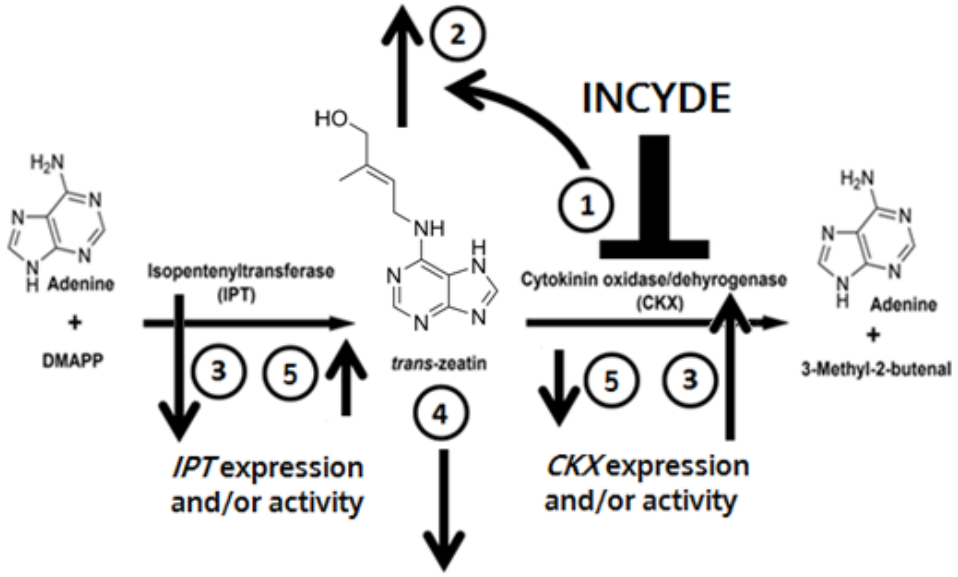

Figure 2.4 The feedback model for the cytokinin response to INCYDE treatment. 1: INCYDE inhibits CKX; 2: This leads to an increase in cytokinin (such as *t*Z); 3: a feedback response is activated involving upregulating *CKX* and/or downregulating *IPT*. 4: The concentration of cytokinin reduces and; 5: results in an corrective decrease in *CKX* and/or increase in *IPT* to normalise cytokinin levels.

2.4.4 The effect of TDZ-K application

TDZ-K is known to inhibit senescence in wheat and barley in detached leaf assays, and not inhibit arabidopsis and wheat root growth (United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript). There was no significant effect of TDZ-K on the growth or yield of RCBr following a single application before anthesis (**Table 2.6**), and this aligned with the property of TDZ-K not inhibiting root growth.

2.4.5 Chlorophyll content

The chlorophyll content of the upper and lower leaves of RCBr plants was measured at 48 d after sowing following INCYDE application four times before anthesis (**Table 2.7**). The decrease in chlorophyll content in lower leaves treated with INCYDE, particularly when plants were grown at 5 mM KNO_3, provides evidence of a INCYDE-induced change in the sink-source dynamics, as this more rapid decline in chlorophyll (the source) coincides with an increase in the seed number (sink) in plants grown with 5 mM KNO_3 (**Table 2.3**), with this enhanced yield being a stronger drain on leaves (source). However, it is also important to note that, even though spray application was focused on the leaf, it was still technically a whole plant application, and despite the results here, the effect of cytokinin enhancement did not necessarily favour a specific part of the plant (or a sink or source).

Unsurprisingly, optimal nitrate nutrition correlated with a greater retention of chlorophyll in both the upper and lower leaves, whereas nitrogen-limited conditions (0.1 mM KNO_3) resulted in total senescence or death of the older leaves. A correlation between chlorophyll content and nitrogen is already well-established (Bojović and Marković, 2009).

Experiments measuring the chlorophyll content of leaves following foliar TDZ-K application, where leaves were acquired at 48 d after sowing (following application before flowering) were not completed, as they were discontinued after preliminary experiments (data not shown) indicated no difference between the control and TDZ-K-treated group. The lack of evidence in preliminary experiments to suggest an inhibition of senescence contrasts with previous reports of TDZ-K inhibiting senescence in detached wheat and barley leaf bioassays (Nisler *et al*., unpublished manuscript), although this inhibition was previously stated to occur only for wheat, and no data was provided for *Brassica* spp. or any other dicots (unpublished data, Palacký University).

2.4.6 Summary

Growth room experiments with RCBr provided limited insight into the efficacy of INCYDE and TDZ-K treatments. Although INCYDE enhanced the number of seeds per plant in RCBr (**Table 2.3**), the effect was specific only to plants grown under 5 mM KNO_3 and when INCYDE was applied four times before flowering. Aside from this yield enhancement, the effects did not ultimately align with the broad range of effects on growth and yield previously reported (unpublished data, Palacký University). The dose-dependent effect of INCYDE on yield led to the proposal of a feedback response mechanism (section **2.4.3**) to normalise endogenous cytokinin levels, which is explored further with gene expression analyses (Chapter 5). The enhancement of yield coincided with a decline in the content of chlorophyll in the lower leaves of plants (**Table 2.7**), as the yield was increased, more nutrients were translocated from the leaves leading to a reduction in the content of chlorophyll relative to the control.

The application of TDZ-K did not result in any changes to growth, yield or chlorophyll content, and while the lack of root inhibition aligned with previous reports of no root inhibition demonstrated in both monocots and dicots, preliminary chlorophyll measurements with TDZ-K did not align with its purported anti-senescence effects (J. Nisler, personal communication, August 28, 2017; United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript). The lack of effect with TDZ-K may indicate that either this compound has very little effect on the growth or development of RCBr or more experimental work is needed to fully evaluate the efficacy of TDZ-K. The effect of each PGR was explored under field and pot (and stressed) conditions with wheat and barley in Chapters 3 and 4 to establish if an enhancement of yield by INCYDE could be replicated, and whether the reported senescence-inhibition of wheat leaves could be demonstrated with TDZ-K.

Chapter 3
Field trials with wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.)

3.1 Introduction

3.1.1 Cereals wheat and barley

Common wheat (*Triticum aestivum* L.) is a major hexaploid cereal crop used globally for human and livestock consumption. In the 2016/17 season it was estimated that 754 million tonnes of wheat were produced globally (International Grains Council, 2017). Even though it has had its genome sequenced recently (https://www.wheatgenome.org/), much remains to be known of the molecular and genetic mechanisms underpinning yield (Van Camp, 2005; Song *et al*., 2012).

Barley (*Hordeum vulgare* L.), is an important cereal crop belonging to the same family as wheat (Poaceae). In 2014/15 136 million tonnes were produced (Matny, 2015), most of it used for animal feed, beverages and as an ingredient for food products (Blake *et al*., 2012). Barley is diploid, and has had a partly ordered draft sequence assembly constructed (International Barley Genome Sequencing Consortium, 2012) which has been fundamental for genetic research.

3.1.2 INCYDE, TDZ-K and CPPU in field trials

One approach to enhancing and manipulating endogenous cytokinin has been to apply cytokinin, such as BA and kinetin, exogenously. There are reports of exogenous cytokinin application affecting grain yield in wheat (Warrier *et al*., 1987; Wang *et al*., 2001; Gupta *et al*., 2003) and barley (Williams and Cartwright, 1980; Hosseini *et al*., 2008). The effects of manipulating cytokinin using this approach are, however, often inconsistent and complex (Koprna *et al*., 2016) and it is difficult to successfully replicate yield enhancements observed in controlled experiments in the field (Nagel *et al*., 2001). Given these limitations, cytokinins are not currently applied to cereals in the field (Jameson and Song, 2016).

The use of compounds that inhibit CKX has been suggested as a more effective approach to enhance endogenous cytokinin (Gemrotová *et al*., 2013; Nisler *et al*., 2016). The use of TDZ and CPPU in the

field has been met with some success: thidiazuron is used as a cotton defoliant (Arndt *et al*., 1976), while CPPU is used to increase the size of fruits including kiwifruit, apples and grapes (Biasi *et al*., 1991; Stern *et al*., 2003; Ferrara *et al*., 2014) following application around 40 µM, applying CPPU in the field with cereals has, however, only had limited success (Jameson and Song, 2016, and references therein). Given that CPPU was applied in these previous experiments in the 10-100 µM concentration range, this concentration range was used for field trials.

Novel PGR INCYDE inhibits CKX more strongly than TDZ (Zatloukal *et al*., 2008), and although there are no published reports of the effect of INCYDE on cereals in field trials, INCYDE was reported to enhance yield in arabidopsis, and winter rapeseed under field conditions, when applied at concentrations of 10 and 50 µM (unpublished data, Palacký University). A biphasic response was demonstrated with INCYDE in *Rumex crispus* and *Bulbine natalensis* seedlings (where seeds germinated in water containing 10 µM INCYDE) (Gemrotová *et al*., 2013), and in arabidopsis plants following root drenching in solutions containing 10 to 100 nM INCYDE (unpublished data, Palacký University). For field and pot trials, a concentration range of 10 to 50 µM was recommended (personal communication, May 22, 2012).

Yield or sink-source dynamics have been altered in cereals by manipulating endogenous cytokinin at a number of different developmental stages. These include as early as targeting seeds pre-sowing (Jafar *et al*., 2012; Afzal *et al*., 2013), following sowing (Criado *et al*., 2009), during tillering, stem elongation, booting, (Yasmeen *et al*., 2013) and ear emergence (Alizadeh *et al*., 2010). There is also good gene expression evidence to suggest targeting cereals during inflorescence development (Yamburenko *et al*., 2017). Anthesis (GS 61-69) is an important growth stage that has been targeted in wheat (Sivakumar *et al*., 2001; Gupta *et al*., 2012) and barley (Hosseini *et al*., 2008). In wheat cultivars, cytokinin levels and activity are known to increase transiently post-anthesis (Wheeler, 1972; Jameson *et al*., 1982; Morris *et al*., 1993; Banowetz *et al*., 1999a; 1999b), while in barley, cytokinin *c*Z appears to predominate early in grain development and *t*Z increases later when grain filling occurs (Powell *et al*., 2013). The increase in cytokinin is required for grain development in cereals (Michael and Seiler-Kelbitsch, 1972; Wheeler, 1972; Jameson *et al*., 1982; Jones *et al*., 1992; Lur and Setter, 1993; Morris *et al*., 1993; Cheikh and Jones, 1994; Dietrich *et al*., 1995; Banowetz *et al*., 1999a; 1999b; Yang *et al*., 2002; Brugière *et al*., 2008). Notably, this increase in cytokinin in wheat coincides with a sharp increase in *TaIPT*s and *TaCKX*s post-anthesis (Song *et al*., 2012), while in barley, there is an elevation of expression of some *HvCKX*s post-anthesis as the kernel develops (Zalewski *et al*., 2014). The increase in *CKX* expression during this period where cytokinin accumulation is critical,

underlined anthesis as an ideal target for CKX-inhibiting INCYDE and CPPU. This contrasts with the finding for RCBr in Chapter 2, where targeting before anthesis was required for yield enhancement. There is no published evidence suggesting a yield enhancement following INCYDE application with cereals, given this, and the limited success with CPPU application in field trials (Jameson and Song, 2016, and references therein) along with the previously described CKX expression patterns observed around anthesis in wheat (Song *et al*., 2012) makes the case of focusing application around anthesis for wheat and barley, as opposed to earlier growth stages in RCBr.

TDZ-K is reported to increase the retention of chlorophyll (at concentration ranges between 0.1 to 100 µM) in wheat and barley leaves under dark (and with wheat dark and light) conditions with detached leaf assays (Nisler *et al*., unpublished manuscript). TDZ-K does this by preventing degradation of photosynthetic complexes in photosystem II (J. Nisler, personal communication, August 28, 2017). This retention of chlorophyll has not be reported for dicots, and was not observed in preliminary experiments carried out with RCBr in Chapter 2. TDZ-K does not show a biphasic response at the concentration range tested with tobacco callus growth (1 to 100 µM) (Nisler *et al*., unpublished manuscript). TDZ-K was targeted towards anthesis and early senescence as these growth stages marked periods early in senescence where TDZ-K might have the most impact on the photosynthetic complexes in photosystem II (J. Nisler, personal communication, August 28, 2017). Based on the concentration ranges described in these experiments, TDZ-K was applied between 10 and 50 µM.

An enhancement of cytokinin is also known to delay senescence, shown particularly in transgenic *IPT*-overexpressing plants, with the delay in senescence mitigating the effect of stress in a variety of crops (Rivero *et al*., 2007; Peleg *et al*., 2011; Qin *et al*., 2011; Guo and Gan, 2014, and references therein). Therefore, enhancing endogenous cytokinin with CKX-inhibiting INCYDE and CPPU was also proposed as an approach for delaying senescence.

The method of application was another important consideration in the field and pot trials. Various experiments have potentially disrupted cytokinin homeostasis by exogenous application of cytokinin with cereals including through irrigation (Williams and Cartwright, 1980; Alizadeh *et al*., 2010), direct injection into specific parts of the plant (Warrier *et al*., 1987; Gupta *et al*., 2003), seed priming with cytokinins (Afzal *et al*., 2013) and spraying (Hosseini *et al*., 2008; Gupta *et al*., 2012; Yasmeen *et al*., 2013). Given the logistics and practical requirements of dealing with treating plants in a farmer's field, and targeting specific growth stages, spraying was considered the only logical approach for applying the PGRs.

3.1.3 Experimental aims

With the insight gained from growth room experiments (Chapter 2), where a yield enhancement from INCYDE application was only evident under very specific growth and application conditions, in addition to the body of research available on field trials and manipulating cytokinin, three field trials were carried out, one (wheat trial) in the 2013/14 season and two (one wheat and barley trial) in the 2014/15 season. The efficacy and effect of applying each PGR on wheat and barley was determined by focusing on four growth stages: the end of elongation, head emergence, anthesis and post-anthesis during senescence, with a significant focus on anthesis. By targeting these growth stages, it was possible to determine the effects of each PGR on growth, yield and grain composition with INCYDE and TDZ-K. The capacity of TDZ-K to inhibit senescence was determined by targeting TDZ-K at senescence in wheat and barley.

3.2 Materials and Methods

3.2.1 Field trials

Three field trials were carried out across two seasons from 2013/14 and 2014/15 at a farm near Lincoln, Canterbury, New Zealand (43°36'15.7"S 172°25'56.0"E and 43°37'04.7"S 172°27'09.4"E). The first season involved autumn feed wheat (*Triticum aestivum* L.) cultivar Orator (PGG Wrightson Grain), which was sown at a rate of 90 kg/ha (April 2013). A second season involved sowing feed barley (*Hordeum vulgare* L.) cultivar Quench (PGG Wrightson Grain) at 115 kg/ha (May 2014) and autumn sown feed wheat cultivar Torch (PGG Wrightson Grain) at 100 kg/ha (September 2014). Plant growth regulators used on the wheat field trials included chlormequat chloride (CCC) (Ravensdown), and Moddus (Syngenta), which are inhibitors of the biosynthesis of gibberellin. While initial discussions with FAR focussed on applying the PGRs under non-optimised conditions, each trial was managed and optimised by the contracted farmer who did not (or was unable) to ensure the trials were separately managed from the surrounding fields. This meant that fertiliser application was optimised and these previously described PGRs were used during development.
Wheat trials had base fertiliser rates of 400 kg/ha (sulfur super and potassium chloride mix). The barley field trial had a base fertiliser rate of 250 kg/ha (Cropmaster 20 (Ravensdown)). Trials were regularly irrigated to give total volumes of 160 mm for Orator wheat, 225 mm for Torch wheat and 280 mm for barley. Continual maintenance of the field with subsequent applications of fertiliser, herbicides, insecticides, fungicides and PGRs, as well as irrigation rates and harvest dates are summarised in Appendix 3.1.

3.2.2 Treatments

For each field trial, treatments of INCYDE, TDZ-K and CPPU were targeted at different growth stages based on Zadoks scale for cereals (Zadoks *et al*., 1974), at concentrations ranging between 10 to 100 µM. The reasoning for using this concentration range is described in section **3.1.2**, along with other experiments where PGRs were applied within this range. These growth stages included the full unrolling of the flag leaf and appearance of the flag leaf ligule (GS 39), the appearance of the first spikelet from the sheath (GS 51), anthesis (GS 61 to 69) and 13 d after anthesis (daa) for Orator wheat or 15 daa for Torch wheat during senescence. The progress of developmental stages was determined by observing the main stems and determining the growth stage based on where a majority (> 50%) of the main stems were at. Developmental stages were assessed based on the appearance of tillers. The appearance of anthesis and its progression were relatively uniform, with a majority of plants within 4 days of GS 61 (anthesis) when treatments were applied. Due to the rapid progression of anthesis (GS 61-69) and the small variation between plants, some treatments were applied twice at anthesis at GS 61 and GS 65, so that the majority of plants received treatment within a few days of GS 61. For the wheat Orator trial (2013/14), bad weather during anthesis slowed the progression of this growth stage. A summary of the treatments is described in **Table 3.1**.

Table 3.1 The plant growth regulator, concentration and growth stage of application made for the wheat cultivar Orator (2013/14), wheat cultivar Torch (2014/15) and barley cultivar Quench (2014/15) field trials.

Tmt. no.	Wheat cv. Orator (2013/14)	Wheat cv. Torch (2014/15)	Barley cv. Quench (2014/15)
	Treatment	Treatment	Treatment
1	NIL	NIL	NIL
2	INCYDE 10 µM (GS 65)	DMSO Control (GS 51, 61, 65, 65+15d)	DMSO Control (GS 39, 51, 61, 65)
3	INCYDE 25 µM (GS 39, 51, 61, 65)	TDZ-K 10 µM (GS 61, 65, 65+15d)	INCYDE 10 µM (GS 65)
4	INCYDE 25 µM (GS 39)	TDZ-K 50 µM (GS 61, 65, 65+15d)	INCYDE 25 µM (GS 39, 51, 61, 65)
5	INCYDE 25 µM (GS 51)	CPPU 10 µM (GS 61, 65)	INCYDE 25 µM (GS 39)
6	INCYDE 25 µM (GS 61)	CPPU 30 µM (GS 61, 65)	INCYDE 25 µM (GS 51)
7	INCYDE 25 µM (GS 65)	CPPU 100 µM (GS 61, 65)	INCYDE 25 µM (GS 61)
8	INCYDE 50 µM (GS 61)	CPPU 10 µM (GS 51, 65)	INCYDE 25 µM (GS 65)
9	INCYDE 50 µM (GS 65)	CPPU 30 µM (GS 51, 65)	INCYDE 50 µM (GS 61)
10	DMSO Control (GS 39, 51, 61, 65)	CPPU 100 µM (GS 51, 65)	INCYDE 50 µM (GS 65)
11	TDZ-K 10 µM (GS 61, 65, 65+13d)		
12	TDZ-K 25 µM (GS 61, 65, 65+13d)		
13	DMSO Control (GS 61, 65, 65+13d)		

The DMSO control is a mixture of water and DMSO applied at the growth stages described while 'Nil' represented no treatment. Growth stages (GS) are based on Zadoks scale. Days (d) represents the number of days from the last growth stage treated.

INCYDE, TDZ-K (Palacký University) and CPPU were prepared by dissolving the compounds in dimethylsulfoxide (DMSO) (Scharlab) to produce 10 mM stock solutions which were stored at -20°C until use. Working solutions were made immediately prior to application by diluting with water and adding surfactant Yates Sprayfix (Yates) at 0.5% (v/v), as recommended by the supplier. Yates Sprayfix is a commercial surfactant which is useful for field and pot applications. A DMSO control was produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments), water and Yates Sprayfix at 0.5%. The 'Nil' treatment represented no treatment or application of a water-DMSO mix. The application of DMSO controls covered all of the growth stages targeted, as indicated in **Table 3.1**, to ensure that any potential effect of DMSO on growth and yield could be separated from effects of INCYDE, TDZ-K or CPPU.

3.2.3 Treatment application

Treatments were applied by New Zealand Arable using CO_2 pressurised hand-hand plot booms, which utilised four air induction AIXR 110 015 nozzles (at 40 cm spacings), pressurised at 250 kpA to allow for an application rate of 187 L/ha for Orator wheat and 170 L/ha for Torch wheat and barley. This reduction was necessary to accommodate the quantity of PGR that was available. Spray was applied 0.5 to 1 m above cereals, with bias coverage towards the flag leaf and leaf directly below this (primary leaf) (**Figure 3.1**). Treatments were made during dry and non-windy conditions to optimise uptake, and made between 9 and 11 am on treatment dates (described in Appendix 3.1).

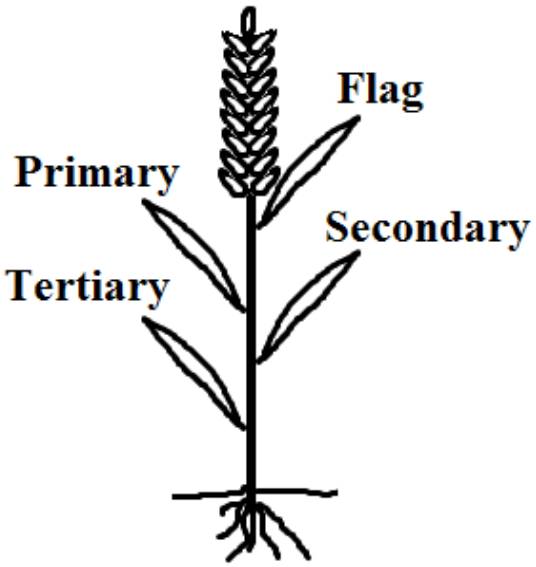

Figure 3.1 The position of flag, primary, secondary and tertiary leaves on wheat and barley.

3.2.4 Experimental design and analyses

The trial was based on a randomised complete block design, with four blocks, each made up of 10-13 plots, with each plot representing a single treatment level replicate (**Figure 3.2**). Each treatment level was represented once in each block. Each block and individual plot was separated by a buffer zone to ensure independence and prevent treatment crossover. The plot size for Orator wheat of the 2013/14 season was 11 x 2.5 m, reduced to 10 m at harvest, while the 2014/15 wheat and barley plots were 10 x 2.5 m.

3.2.4.1 New Zealand Grainlab harvesting and analyses

Separate analyses were carried out by the New Zealand Grainlab (Canterbury) to determine the yield and protein composition using industrial methods and standards. When wheat and barley plants had undergone complete senescence, samples were harvested and analysed. Analysis was carried out by harvesting the plots using a Sampo combine harvester (Sampo Rosenlew Ltd) with a moisture probe and on-board weight which calculated the yield in T/ha. For each plot, using 20 g of screened grain samples, the thousand grain weight (TGW) was calculated with a Numigral I seed counter (Sinar). The protein composition was calculated using an Instalab® 700 NIR Analyzer (DICKEY-john). For the New Zealand Grainlab analyses, the mean TGW, T/ha and protein composition was calculated for the plots from each of the four blocks, giving four replicate means (each generated from this previously described subsample) in total for each treatment level (**Figure 3.2**). For each treatment level, the overall mean was calculated using these four replicate means, and the overall mean and standard error was reported in the tables of the results sections.

To determine if there were any statistically significant differences between the treatment levels, an ANOVA (significance level: 0.05, two sided) was carried out on the treatment replicate means using Minitab 17 (Minitab Inc.). The ANOVA was partitioned for the main factor (the treatment) and the block factor, to determine if either has a significant effect on the data. All factors were fixed. The protein composition data (which is proportional data) was Logit-transformed prior to ANOVA using Microsoft Excel 2016 (Microsoft) according to the following equation:

$$\text{Logit} = \log(p/(1 - p))$$

Where p is the proportion (data input) and log is the natural log. To determine if the residuals were normally distributed to meet the assumption of the ANOVA, a Q-Q plot was examined and reported using the standardised residuals. The ANOVA assumption of equality of the variances was determined by plotting the standardised residuals against predicted values and determining the distribution around 0. Both of these residual plots were produced in Minitab 17 and reported in the Appendices indicated in each results table. As no *p*-value in these analyses was ≤ 0.05 for the treatment factor, no *post hoc* tests were carried out with the Grainlab data.

3.2.4.2 University-based laboratory harvesting, subsampling and analyses

A second analysis was carried out at the University of Canterbury. For each treatment level, ten whole wheat or barley plants were acquired from each plot within each of three blocks (**Figure 3.2**). From the subsample of ten plants, the number of tillers present in each plant was recorded and the mean tiller number calculated for each plot. The overall mean number of tillers was calculated for a treatment level (using the three replicates) and the overall mean reported with the standard error in the results tables. To calculate if there was any statistically significant difference between a treatment and the controls, a Poisson regression (log-link function, confidence interval: 95%, Two-sided) using Minitab 17 was carried out, with the DMSO control set as the reference level, and the coefficients and *p*-values were examined to determine if there was a statistically significant difference between the DMSO control and treatments.

Using the ten whole plants taken from each plot, individual tillers were divided into large/main stems and small/tiller stems (**Figure 3.2**). From the separate pool of large and small tillers, a subsample of ten large and small tillers was used to measure the stem length, stem dry weight (DW), stem diameter, root DW, head length and head DW. The shoot length was measured as the length from the point of connection to the head to the soil line (this section was also used to measure the stem DW). The stem diameter was measured at the first node above the soil line. The head length was measured, from the point of connection to the stem to the highest point of the upper most spikelet. The head DW was measured following excision of head at this point of connection. The root DW measurements were made, with the root DW defined as the root segment attached to each tiller that was below the soil line. This is not the root of the entire plant but the root segment of a single tiller. All samples that were measured for dry weight (DW) and subsequent yield measurements were air dried for two weeks and then placed in ovens for 2 h at 70 to 80°C until weight stabilisation immediately prior to measurements.

For wheat cultivar Orator, for each plot, five large and five small heads were taken to calculate the percentage of floret positions filled by grains (**Figure 3.2** and **3.3**). For thousand grain weight (TGW) measurements, ten large and ten small heads were taken from each plot, and the wheat and barley heads were divided into third sections with the bottom third defined as the part of the head attached to the stem (**Figures 3.3A** and **B**). In wheat, each spikelet was divided into five different possible floret positions where wheat grains can develop, with positions 1 and 2 containing the heaviest grains and positions 3 to 5 containing smaller grains that were less likely to develop (**Figure 3.3C**). Grains were

removed from each third section in wheat and barley and additionally each floret position in wheat. A random sample of thirty grains was taken from each of these different positions and the TGW was measured and recorded for the plot. The number of samples taken from each replicate for each treatment level was equal. For each of the growth and yield traits described, a mean was generated for each replicate of a treatment level (individual plot) and an overall mean was generated for each treatment level using the three replicate means and reported along with the standard error in the results tables.

For the growth and yield traits, an ANOVA (significance level: 0.05, two sided) was carried out to determine whether there was a statistically significant difference between the means, using Minitab 17. The ANOVA was partitioned for the main factor (treatments) and the block factor. All factors were fixed. The *F*-values and *p*-values were reported in the results section. The % grain filling data was Logit-transformed using Microsoft Excel 2016 (as described previously) before ANOVA. The assumption of equality of the variances and residuals being normally distributed was demonstrated with residual plots using Minitab 17 and reported in the Appendix. In the case where there was a *p*-value ≤ 0.05 for the treatment factor, a *post hoc* Tukey HSD (confidence interval: 95%) test was used to make pairwise comparisons and determine if there were statistically significant differences between means for different factors using XLSTAT 2016 (Addinsoft). This is also reported in the Appendix. Based on the Tukey HSD, statistically significant differences were reported in the results tables for treatments (INCYDE, TDZ-K or CPPU) that were significantly different from all of the controls groups, including the DMSO controls and NIL, and this was indicated with an *. A Levene's test (Gastwirth *et al*., 2009) was also carried out and the *p*-value was provided in the notes of the results table for results where there was a statistically significant difference between a treatment and the controls.

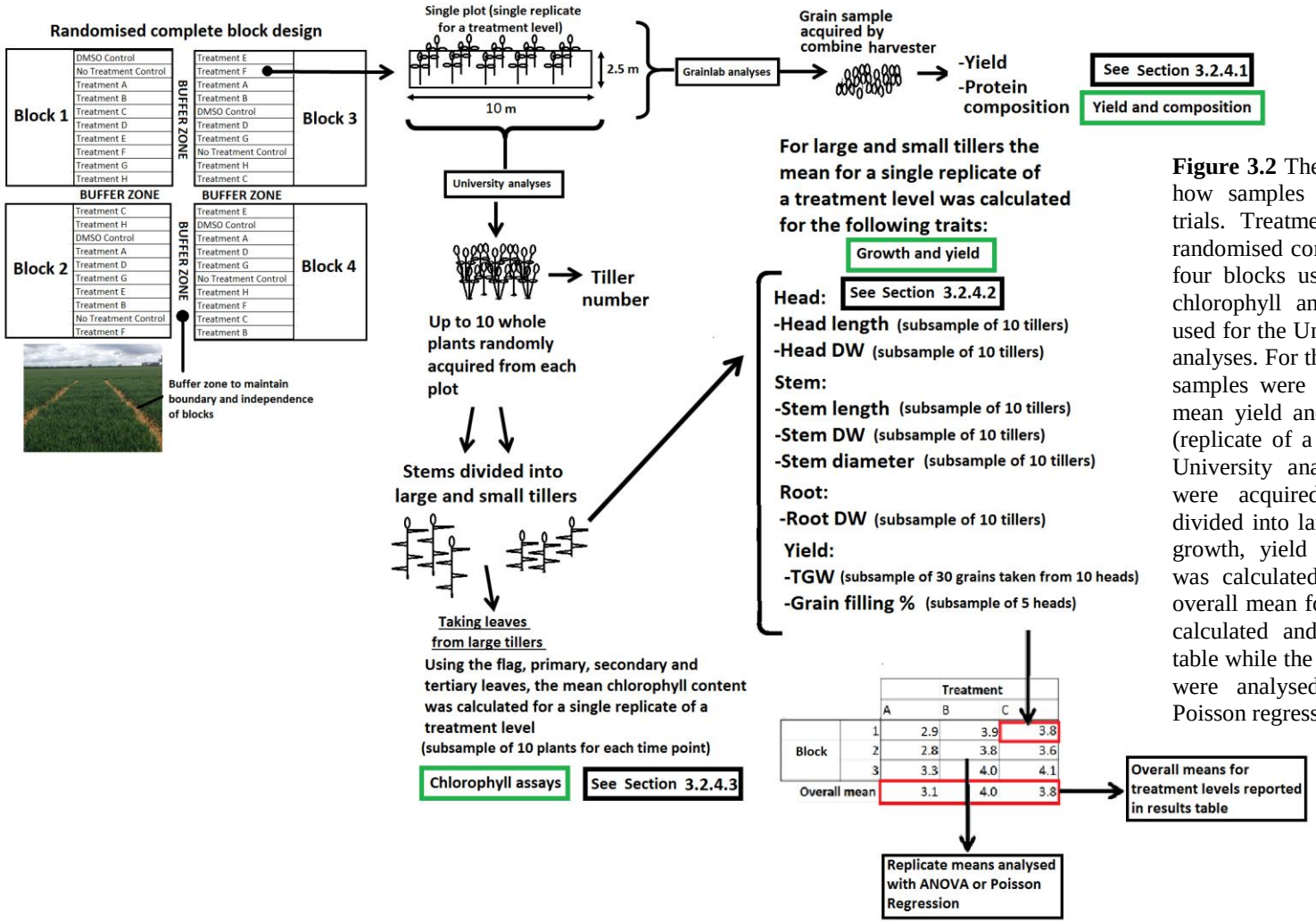

Figure 3.2 The experimental design and how samples were acquired for field trials. Treatments were arranged in a randomised complete block design, with four blocks used for the Grainlab and chlorophyll analyses and three blocks used for the University growth and yield analyses. For the Grainlab analyses, 20 g samples were acquired to calculate the mean yield and composition for a plot (replicate of a treatment level). For the University analyses, ten whole plants were acquired from each plot and divided into large and small tillers. The growth, yield and chlorophyll content was calculated from these tillers. The overall mean for the treatment level was calculated and reported in the results table while the treatment replicate means were analysed with an ANOVA or Poisson regression.

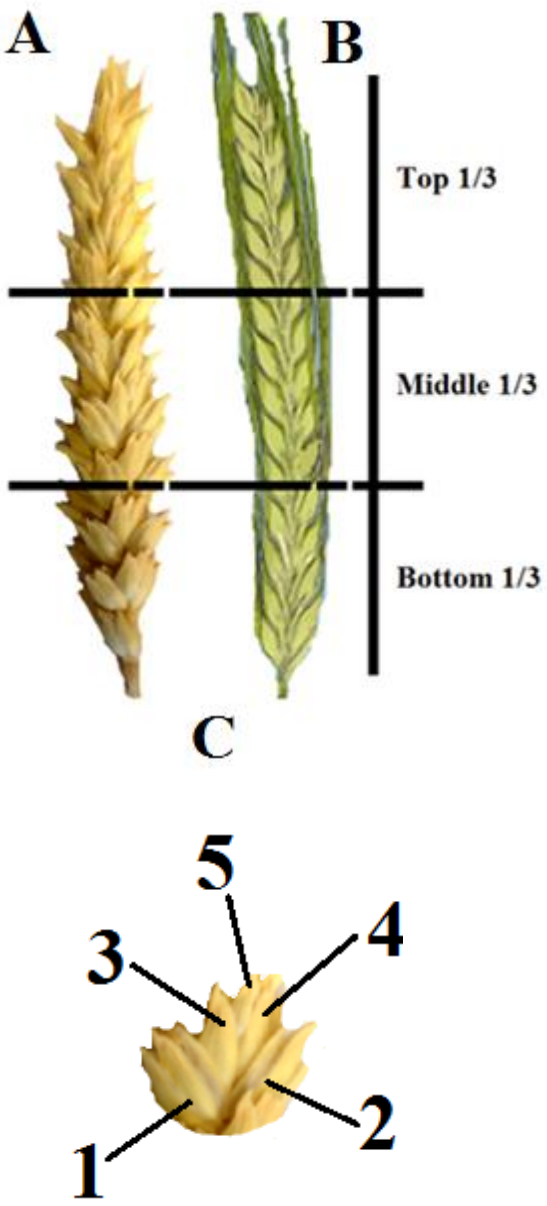

Figure 3.3 The structure of wheat and barley heads. A: Wheat head; B: barley head divided into third sections; C: wheat floret positions 1 to 5.

3.2.4.3 Chlorophyll content analyses

The chlorophyll content was measured following TDZ-K 50 µM (GS 61, 65, 65+15d) and CPPU 100 µM (GS 51, 65) treatments in Torch wheat and INCYDE at 25 µM (GS 39, 51, 61, 65) and 50 µM (GS 61) in barley. For each time point, using ten large/main stems acquired from ten plants from each plot (**Figure 3.2**), the flag, primary, secondary and for barley the tertiary leaves (**Figure 3.1**) were collected after the end of the last treatment, defined as week 0, that is 15 days after GS 65 for wheat, and at GS 65 for barley. Samples were then acquired every two weeks in Torch wheat and every 2.5 weeks in barley. Samples were taken from four blocks (as was the case with the Grainlab analyses) to give a total of four replicates for each treatment level. Leaf samples measuring 1 cm x 1 cm were taken from the centre of wheat and barley leaves.

Each replicate weighed between 3 and 90 mg and was added to 1 mL dimethylforamide (DMF) (Sigma-Aldrich) to produce solutions of no more than 0.09 mg FW/µL DMF, and solutions were analysed using the protocol in Evans *et al*. (2012) described in section **2.2.5**. For each treatment level replicate (plot), the mean chlorophyll content (in mg/g) of the leaves was generated from the subsample of ten plants. The overall mean for each treatment level was calculated from the treatment replicate means and reported along with the standard error in the results tables.

Chlorophyll data was analysed with an ANOVA (significance level: 0.05, two sided) partitioned for the main factor: treatment, the time point when leaf samples were acquired, the interaction between the time and treatment factors (time*treatment) and the block effect. All factors were fixed. The ANOVA was carried out using Minitab 17, and the *F*-value and *p*-values reported for each factor of interest in the results tables. Residual plots were carried out with Minitab 17 to determine whether residuals were normally distributed, and whether the variances were homoscedastic, and reported in the Appendix described in the result tables. Where the ANOVA showed that the *p*-value for a factor or the interaction was ≤ 0.05, a *post hoc* Tukey HSD test (confidence interval: 95%) was carried out to determine if there were statistically significant differences between a treatment and the control (XLSTAT 2016). Where there was evidence for an a time*treatment interaction effect ($p \leq 0.05$), an interaction plot was reported in the Appendix (XLStat 2016). The effect of treatments overall and at specific time points were both determined. Based on the Tukey HSD pairwise comparisons, where a significant difference existed between the control and a treatment at a specific time point, an * was provided next to the chlorophyll content value to indicate this. Where there was statistically significant difference between a treatment group and the control group (e.g. a pairwise comparison of

INCYDE vs. control), a ** was placed next to the respective treatment. The interpretation of statistically significant effects of main factors (and whether it was appropriate to interpret main effects) depended on the presence of an interaction effect and the interaction plots.

3.2.5 Vegetation greenness

Vegetation greenness was measured before and after treatments in Orator wheat, and before treatments in Torch wheat and barley, to ensure there were no differences between designated controls and treatments. Two indices were calculated using the GreenSeeker® (NTech), the Normalised Difference Vegetative Index (NDVI or NVI) and the Inverse Ratio Vegetative Index (IRVI) ratio. These indices compare the relative reflectance of soils and plant material using visible wavelengths (red) (R_{VIS}) at 660 nm and near infrared (R_{NIR}) at 770 nm with the following equations:

$$\mathbf{NDVI = (R_{NIR} - R_{VIS})/(R_{NIR} + R_{VIS})}$$

$$\mathbf{IRVI = R_{VIS}/R_{NIR}}$$

The NDVI was calculated for each plot, with four plots (replicates) measured for each treatment level. The overall mean NDVI was reported for each treatment level and reported with the standard error in Appendix 3.2. In order to determine if there were any differences in the means for NDVI between each treatment level, the data which was proportional, was Logit-transformed and analysed with an ANOVA partitioned for the treatment and block factors.

3.2.6 Biotic stress

Following a significant period of rain in 2013 it was evident that Orator wheat was infected by *Septoria tritici* blotch. To measure and determine if INCYDE or TDZ-K treatments conveyed resistance to this pathogen, for each treatment level replicate (plot) nine primary leaves were randomly collected from nine different plants and the mean proportion of fungal-induced senescence was measured for this plot. This was repeated for two more replicates (plots) for each treatment level, and the overall mean was calculated and reported for the three replicates of a treatment level. As with other proportion data, the data was Logit-transformed before being analysed with ANOVA to determine the difference in the means between treatment levels.

3.3 Results

3.3.1 Vegetation greenness

Vegetative greenness was determined using GreenSeeker® before treatment application to ensure there were no differences between the plots and blocks as a result of extraneous factors such as the environment. These results are presented in Appendices 3.2.1 and 3.2.2. A measurement was made after treatments in wheat Orator as this field trial was subject to poor weather and infected with *Septoria tritici*, and it remained to be determined if greenness was affected more in some blocks or plots than others. Analyses of variance of the vegetation greenness showed that there were no statistically significant differences in the mean greenness of treatment levels in wheat cultivar Orator both prior ($F_{12, 36}$ = 1.88, p = 0.071, Appendix 3.2.1) and after ($F_{12, 36}$ = 0.82, p = 0.628) treatment application. Likewise, ANOVA revealed that there were no significant differences between the means prior to treatment application in wheat cultivar Torch ($F_{9, 27}$ = 1.3, p = 0.28, Appendix 3.2.2) or barley cultivar Quench ($F_{9, 27}$ = 2.09, p = 0.067).

3.3.2 New Zealand Grainlab analyses of yield and protein

New Zealand Grainlab were independently carried out to determine the yield and grain composition using industrial methods and standards. Using data produced by the New Zealand Grainlab, ANOVA revealed that for wheat cultivar Orator (**Table 3.2**), there was no statistically significant difference between the means in the yield (T/ha) ($F_{12, 36}$ = 1.89, p = 0.069, Appendix 3.3.1), TGW ($F_{12, 36}$ = 0.89, p = 0.569, Appendix 3.3.2) or protein composition ($F_{12, 36}$ = 1.21, p = 0.316, Appendix 3.3.3).

Table 3.2 New Zealand Grainlab analyses of yield, thousand grain weight (TGW) and protein content in wheat cultivar Orator (2013/14) following INCYDE and TDZ-K treatment.

Treatment	Yield (T/ha)	TGW (g)	Protein (%)
Nil	10.8 ± 0.2	46.0 ± 1.1	11.2 ± 0.2
DMSO Control (GS 39, 51, 61, 65)	11.1 ± 0.1	46.6 ± 0.2	11.2 ± 0.1
DMSO Control (GS 61, 65, 65+13d)	11.2 ± 0.1	47.2 ± 0.7	11.0 ± 0.1
INCYDE 10 µM (GS 65)	11.4 ± 0.2	45.9 ± 1.1	11.4 ± 0
INCYDE 25 µM (GS 39, 51, 61, 65)	11.3 ± 0.2	46.2 ± 0.7	11.1 ± 0.1
INCYDE 25 µM (GS 39)	11.2 ± 0.2	45.0 ± 1.1	11.2 ± 0.1
INCYDE 25 µM (GS 51)	11.0 ± 0.1	45.4 ± 0.8	11.1 ± 0.2
INCYDE 25 µM (GS 61)	11.1 ± 0.2	45.6 ± 0.4	11.1 ± 0.1
INCYDE 25 µM (GS 65)	11.1 ± 0.2	45.1 ± 0.5	11.2 ± 0.1
INCYDE 50 µM (GS 61)	11.1 ± 0.1	45.5 ± 1.2	11.4 ± 0.1
INCYDE 50 µM (GS 65)	11.1 ± 0.2	46.9 ± 0.3	11.4 ± 0.1
TDZ-K 10 µM (GS 61, 65, 65+13d)	11.1 ± 0.1	46.3 ± 1.1	11.1 ± 0.1
TDZ-K 25 µM (GS 61, 65, 65+13d)	11.4 ± 0.1	46.0 ± 0.5	11.1 ± 0.1
Treatment *F*-value ($F_{12, 36}$)[a]	1.89	0.89	1.21
Treatment *p*-value	0.069	0.569	0.316
Statistics	**Appendix 3.3.1**	**Appendix 3.3.2**	**Appendix 3.3.3**
Analysis notes	ANOVA	ANOVA	Logit-transformed, ANOVA

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).
The values provided are overall means for four independent replicates (plots) (n = 4), presented with the standard error. Each plot is represented once in each block, arranged in a randomised complete block design. For each plot, analyses were carried out by the NZ Grainlab using 20 g of screened grain samples as described in section **3.2.4.1**. The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **3.2.2**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA and residual plots for the ANOVA assumptions are provided in **Appendices 3.3.1** to **3.3.3** for each trait, and both *F*-values and *p*-values are provided. Protein percentage data was logit-transformed prior to ANOVA. For more information on the experimental design and analyses see section **3.2.4**.

For wheat cultivar Torch (**Table 3.3)**, ANOVA revealed that there was no significant difference between the means in the yield (T/ha) ($F_{9, 27}$ = 0.46, p = 0.891, Appendix 3.3.4), TGW ($F_{9, 27}$ = 1.46, p = 0.214, Appendix 3.3.5) or protein composition ($F_{9, 27}$ = 1.27, p = 0.295, Appendix 3.3.6). Likewise, for the barley cultivar Quench (**Table 3.4**) there is no significant difference between the means in the yield (T/ha) ($F_{9, 27}$ = 1.12, p = 0.385, Appendix 3.3.7), TGW ($F_{9, 27}$ = 0.37, p = 0.938, Appendix 3.3.8) and protein composition ($F_{9, 27}$ = 0.28, p = 0.975, Appendix 3.3.9).

Table 3.3 New Zealand Grainlab analyses of yield, thousand grain weight (TGW) and protein content in wheat cultivar Torch (2014/15) following TDZ-K and CPPU treatment.

Treatment	Yield (T/ha)	TGW (g)	Protein (%)
Nil	14.5 ± 0.1	46.8 ± 1.1	9.8 ± 0.2
DMSO Control (GS 51, 61, 65, 65+15d)	14.5 ± 0.3	48.1 ± 0.2	9.9 ± 0.2
TDZ-K 10 µM (GS 61, 65, 65+15d)	14.5 ± 0.2	47.4 ± 0.5	10.0 ± 0.2
TDZ-K 50 µM (GS 61, 65, 65+15d)	14.7 ± 0.3	49.1 ± 0.5	9.8 ± 0.05
CPPU 10 µM (GS 61, 65)	14.4 ± 0.3	47.1 ± 0.4	10.0 ± 0.2
CPPU 30 µM (GS 61, 65)	14.5 ± 0.3	48.8 ± 0.2	9.7 ± 0.1
CPPU 100 µM (GS 61, 65)	14.6 ± 0.2	48.4 ± 0.3	9.7 ± 0.3
CPPU 10 µM (GS 51, 65)	14.5 ± 0.2	47.8 ± 0.4	9.6 ± 0.1
CPPU 30 µM (GS 51, 65)	14.7 ± 0.1	47.2 ± 0.9	9.9 ± 0.1
CPPU 100 µM (GS 51, 65)	14.4 ± 0.2	48.1 ± 0.8	9.9 ± 0.2
Treatment *F*-value ($F_{9, 27}$)[a]	0.46	1.46	1.27
Treatment *p*-value	0.891	0.214	0.295
Statistics	**Appendix 3.3.4**	**Appendix 3.3.5**	**Appendix 3.3.6**
Analysis notes	ANOVA	ANOVA	Logit-transformed, ANOVA

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

The values provided are overall means for four independent replicates (plots) (n = 4), presented with the standard error. Each plot is represented once in each block, arranged in a randomised complete block design. For each plot, analyses were carried out by the NZ Grainlab using 20 g of screened grain samples as described in section **3.2.4.1**. The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **3.2.2**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA and residual plots for the ANOVA assumptions are provided in **Appendices 3.3.4** to **3.3.6** for each trait, and both *F*-values and *p*-values are provided. Protein percentage data was logit-transformed prior to ANOVA. For more information on the experimental design and analyses see section **3.2.4**.

Table 3.4 New Zealand Grainlab analyses of yield, thousand grain weight (TGW) and protein content in barley cultivar Quench (2014/15) following INCYDE treatment.

Treatment	Yield (T/ha)	TGW (g)	Protein (%)
Nil	10.8 ± 0.2	52.3 ± 0.5	14.3 ± 0.1
DMSO Control (GS 51, 61, 65, 65+15d)	11.0 ± 0.1	52.0 ± 0.4	13.9 ± 0.4
INCYDE 10 µM (GS 65)	11.0 ± 0.2	53.0 ± 0.2	13.8 ± 0.3
INCYDE 25 µM (GS 39, 51, 61, 65)	11.2 ± 0.2	51.8 ± 0.5	14.0 ± 0.2
INCYDE 25 µM (GS 39)	11.3 ± 0.2	52.5 ± 0.5	13.8 ± 0.2
INCYDE 25 µM (GS 51)	11.3 ± 0.1	52.4 ± 0.9	14.0 ± 0.4
INCYDE 25 µM (GS 61)	11.1 ± 0.1	52.2 ± 0.9	13.9 ± 0.5
INCYDE 25 µM (GS 65)	11.3 ± 0.1	52.5 ± 0.7	14.2 ± 0.4
INCYDE 50 µM (GS 61)	11.2 ± 0.1	52.5 ± 0.8	14.1 ± 0.4
INCYDE 50 µM (GS 65)	11.2 ± 0.3	52.3 ± 0.4	13.9 ± 0.5
Treatment *F*-value ($F_{9, 27}$)[a]	1.12	0.37	0.28
Treatment *p*-value	0.385	0.938	0.975
Statistics	**Appendix 3.3.7**	**Appendix 3.3.8**	**Appendix 3.3.9**
Analysis notes	ANOVA	ANOVA	Logit-transformed, ANOVA

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

The values provided are overall means for four independent replicates/plots (n = 4), presented with the standard error. Each plot is represented once in each block, arranged in a randomised complete block design. For each plot, analyses were carried out by the NZ Grainlab using 20 g of screened grain samples as described in section **3.2.4.1**. The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **3.2.2**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA and residual plots for the ANOVA assumptions are provided in **Appendices 3.3.7** to **3.3.9** for each trait, and both *F*-values and *p*-values are provided. Protein percentage data was logit-transformed prior to ANOVA. For more information on the experimental design and analyses see section **3.2.4**.

3.3.3 Orator wheat yield, dry weight and growth

The first field trial, carried out in the 2013/14 season with wheat cultivar Orator, which focused on the effect of novel PGRs INCYDE and TDZ-K with focus on targeting anthesis and early senescence with a concentration range between 10 and 50 µM. The reasoning for targeting anthesis and using this concentration range is described in section **3.1.2**. For the wheat cultivar Orator, there is no statistically significant difference between the means for the tiller number (Poisson regression: p = 1.0, Appendix 3.4.7) (**Table 3.5**). Analyses of variances revealed no statistically significant difference between the means in the length of heads of large tillers ($F_{12, 24}$ = 1.57, p = 0.167, Appendix 3.4.1) and small tillers ($F_{12, 24}$ = 0.72, p = 0.719, Appendix 3.4.2), the DW of heads of large tillers ($F_{12, 24}$ = 1.14, p = 0.376, Appendix 3.4.3) and small tillers ($F_{12, 24}$ = 1.11, p = 0.394, Appendix 3.4.4), the length of stems of large tillers ($F_{12, 24}$ = 1.11, p = 0.399, Appendix 3.4.5) and small tillers ($F_{12, 24}$ = 1.06, p = 0.432, Appendix 3.4.6). ANOVA also reveal that there is no statistically significant difference between the means in wheat cultivar Orator for other growth traits including the stem DW of large tillers ($F_{12, 24}$ =

2.06, p = 0.063, Appendix 3.4.8) (**Table 3.6**) and small tillers ($F_{12, 24}$ = 1.08, p = 0.416, Appendix 3.4.9), the root DW of large tillers ($F_{12, 24}$ = 0.72, p = 0.717, Appendix 3.4.10) and small tillers ($F_{12, 24}$ = 1.14, p = 0.373, Appendix 3.4.11), the stem diameter of large tillers ($F_{12, 24}$ = 1.53, p = 0.181, Appendix 3.4.12) and small tillers ($F_{12, 24}$ = 0.51, p = 0.888, Appendix 3.4.13).

Table 3.5 The wheat cultivar Orator (2013/14) tiller number, head length, head dry weight and stem length for large and small tillers following INCYDE and TDZ-K treatment.

Treatment	Large Head Length (mm)	Small Head Length (mm)	Large Head DW (g)	Small Head DW (g)	Large Stem Length (mm)	Small Stem Length (mm)	Tiller no.
Nil	99.5 ± 0.9	87.2 ± 1.7	3.1 ± 0.1	2.0 ± 0.1	715.1 ± 6.0	676.1 ± 9.1	3.7 ± 0.2
DMSO Control (GS 39, 51, 61, 65)	99.4 ± 2.0	93.9 ± 6.3	3.1 ± 0.2	2.4 ± 0.3	732.3 ± 11.4	703.6 ± 10.1	3.6 ± 0.1
DMSO Control (GS 61, 65, 65+13d)	98.4 ± 1.0	88.1 ± 4.4	3.2 ± 0.2	2.2 ± 0.3	700.5 ± 3.3	678.8 ± 5.9	3.8 ± 0.3
INCYDE 10 µM (GS 65)	98.3 ± 0.7	88.3 ± 2.2	2.9 ± 0.1	2.0 ± 0.1	719.3 ± 14.6	700.6 ± 8.3	4.0 ± 0.3
INCYDE 25 µM (GS 39, 51, 61, 65)	97.8 ± 3.1	92.8 ± 1.8	3.1 ± 0.1	2.2 ± 0.1	697.3 ± 13.0	695.8 ± 5.4	3.4 ± 0.2
INCYDE 25 µM (GS 39)	101.2 ± 0.7	92.1 ± 1.0	3.2 ± 0.2	2.2 ± 0.03	724.9 ± 1.6	696.0 ± 15.8	4.4 ± 0.1
INCYDE 25 µM (GS 51)	104.6 ± 1.1	93.8 ± 0.9	3.2 ± 0.1	2.2 ± 0.1	716.4 ± 9.9	691.8 ± 6.9	3.7 ± 0.2
INCYDE 25 µM (GS 61)	101.2 ± 1.6	88.1 ± 2.2	3.2 ± 0.1	2.0 ± 0.2	705.1 ± 6.5	681.8 ± 17.8	3.8 ± 0.3
INCYDE 25 µM (GS 65)	101.6 ± 2.8	92.7 ± 2.6	3.3 ± 0.2	2.3 ± 0.1	690.4 ± 3.1	680.6 ± 6.4	3.8 ± 0.2
INCYDE 50 µM (GS 61)	105.0 ± 0.2	95.7 ± 1.7	3.2 ± 0.1	2.3 ± 0.2	712.9 ± 21.6	688.8 ± 4.3	3.6 ± 0.1
INCYDE 50 µM (GS 65)	99.7 ± 2.7	90.8 ± 3.3	3.1 ± 0.1	2.0 ± 0.1	715.8 ± 2.7	684.5 ± 5.3	3.5 ± 0.1
TDZ-K 10 µM (GS 61, 65, 65+13d)	101.2 ± 2.1	90.4 ± 2.6	3.5 ± 0.3	2.5 ± 0.03	714.5 ± 8.3	682.9 ± 8.8	3.7 ± 0.3
TDZ-K 25 µM (GS 61, 65, 65+13d)	99.0 ± 2.1	91.6 ± 6.2	3.0 ± 0.1	2.1 ± 0.2	717.6 ± 15.7	669.1 ± 12.6	3.6 ± 0.1
Treatment *F*-value ($F_{12, 24}$)[a]	1.57	0.72	1.14	1.11	1.11	1.06	
Treatment *p*-value	0.167	0.719	0.376	0.394	0.399	0.432	1.0
Statistics	**Appendix 3.4.1**	**Appendix 3.4.2**	**Appendix 3.4.3**	**Appendix 3.4.4**	**Appendix 3.4.5**	**Appendix 3.4.6**	**Appendix 3.4.7**
Analysis notes	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA	Poisson regression

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

For wheat cultivar Orator (2013/14), the overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates/plots (n = 3), within separate blocks, arranged in a randomised complete block design. Analyses were carried out at the University of Canterbury by taking ten whole plants from each plot (30 in total for each treatment level), and dividing plants into large and small tillers as described in section **3.2.4.2** and **Figure 3.2**. The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **3.2.2**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA, Poisson regression and residual plots for the ANOVA assumptions are provided in **Appendices 3.4.1** to **3.4.7** for each trait. *F*-values and *p*-values are provided for non-count data (head length, head DW and stem length) for the treatment factor. For count data (tiller number), a *p*-value is provided for the treatment factor of a Poisson regression. For more information on the experimental design and analyses see section **3.2.4**.

Table 3.6 The wheat cultivar Orator (2013/14) stem dry weight, root dry weight and stem diameter for large and small tillers following INCYDE and TDZ-K treatment.

Treatment	Large Stem DW (g)	Small Stem DW (g)	Large Root DW (g)	Small Root DW (g)	Large Stem Diameter (mm)	Small Stem Diameter (mm)
Nil	1.9 ± 0.1	1.4 ± 0.1	0.14 ± 0.01	0.10 ± 0.003	4.6 ± 0.1	4.4 ± 0.1
DMSO Control (GS 39, 51, 61, 65)	2.0 ± 0.1	1.6 ± 0.1	0.13 ± 0.01	0.09 ± 0.02	4.6 ± 0.03	4.3 ± 0.1
DMSO Control (GS 61, 65, 65+13d)	1.7 ± 0.03	1.3 ± 0.03	0.12 ± 0.01	0.09 ± 0.01	4.7 ± 0.1	4.4 ± 0.1
INCYDE 10 µM (GS 65)	1.9 ± 0.1	1.4 ± 0.03	0.13 ± 0.01	0.08 ± 0.01	4.8 ± 0.1	4.3 ± 0.1
INCYDE 25 µM (GS 39, 51, 61, 65)	1.7 ± 0.1	1.5 ± 0.1	0.14 ± 0.01	0.10 ± 0.02	4.5 ± 0.1	4.3 ± 0.1
INCYDE 25 µM (GS 39)	2.0 ± 0.03	1.6 ± 0.2	0.17 ± 0.01	0.12 ± 0.01	4.7 ± 0.03	4.4 ± 0.03
INCYDE 25 µM (GS 51)	1.9 ± 0.1	1.5 ± 0.1	0.14 ± 0.02	0.12 ± 0.02	4.8 ± 0.03	4.4 ± 0.1
INCYDE 25 µM (GS 61)	1.9 ± 0.1	1.4 ± 0.1	0.13 ± 0.01	0.10 ± 0.01	4.6 ± 0.1	4.3 ± 0.03
INCYDE 25 µM (GS 65)	1.8 ± 0.1	1.6 ± 0.1	0.14 ± 0.02	0.10 ± 0.01	4.5 ± 0.1	4.4 ± 0.03
INCYDE 50 µM (GS 61)	2.0 ± 0.03	1.5 ± 0.1	0.14 ± 0.01	0.11 ± 0.01	4.7 ± 0.1	4.4 ± 0.1
INCYDE 50 µM (GS 65)	1.9 ± 0.1	1.5 ± 0.1	0.12 ± 0.01	0.11 ± 0.003	4.6 ± 0.1	4.3 ± 0.1
TDZ-K 10 µM (GS 61, 65, 65+13d)	1.9 ± 0.1	1.5 ± 0.03	0.15 ± 0.04	0.11 ± 0.01	4.7 ± 0.03	4.4 ± 0.03
TDZ-K 25 µM (GS 61, 65, 65+13d)	1.9 ± 0.1	1.4 ± 0.1	0.14 ± 0.02	0.09 ± 0.003	4.6 ± 0.03	4.3 ± 0.03
Treatment *F*-value ($F_{12, 24}$)[a]	2.06	1.08	0.72	1.14	1.53	0.51
Treatment *p*-value	0.063	0.416	0.717	0.373	0.181	0.888
Statistics	**Appendix 3.4.8**	**Appendix 3.4.9**	**Appendix 3.4.10**	**Appendix 3.4.11**	**Appendix 3.4.12**	**Appendix 3.4.13**
Analysis notes	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

For wheat cultivar Orator (2013/14), the overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates/plots (n = 3), within separate blocks, arranged in a randomised complete block design. Analyses were carried out at the University of Canterbury by taking ten whole plants from each plot (30 in total for each treatment level), and dividing plants into large and small tillers as described in section **3.2.4.2** and **Figure 3.2**. The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **3.2.2**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA and residual plots for the ANOVA assumptions are provided in **Appendices 3.4.8** to **3.4.13** for each trait. *F*-values and *p*-values are provided for stem DW, root DW and stem diameter for the treatment factor. For more information on the experimental design and analyses see section **3.2.4**.

With components of yield in wheat cultivar Orator (**Table 3.7**), ANOVA revealed that there was no statistically significant difference between means in the TGW overall in large tillers ($F_{12, 24}$ = 0.96, p = 0.511, Appendix 3.4.14) and small tillers ($F_{12, 24}$ = 1.18, p = 0.347, Appendix 3.4.15), the TGW at positions 1 and 2 in the heads of large tillers ($F_{12, 24}$ = 2.19, p = 0.05, Appendix 3.4.16) and small tillers ($F_{12, 24}$ = 0.42, p = 0.939, Appendix 3.4.17), nor in the TGW at positions 3 to 5 in the heads of large tillers ($F_{12, 24}$ = 0.88, p = 0.575, Appendix 3.4.18) and small tillers ($F_{12, 24}$ = 0.99, p = 0.483, Appendix 3.4.19).

Although ANOVA reveals a significant effect of the treatments on the TGW of the middle third section of heads of large tillers ($F_{12, 24}$ = 2.44, p = 0.03, Appendix 3.4.22) (**Table 3.8**), a *post hoc* Tukey HSD test did not show any significant difference between a PGR treatment (INCYDE or TDZ-K) and the control groups. Similarly, analysis of variances indicate no statistically significant difference in the TGW at other positions including in the top third section of heads of large tillers ($F_{12, 24}$ = 0.91, p = 0.554, Appendix 3.4.20) and small tillers ($F_{12, 24}$ = 0.69, p = 0.744, Appendix 3.4.21), the middle third sections of heads of large tillers ($F_{12, 24}$ = 2.44, p = 0.03, Appendix 3.4.22) and small tillers ($F_{12, 24}$ = 1.26, p = 0.303, Appendix 3.4.23) and the bottom third sections of heads of large tillers ($F_{12, 24}$ = 2.14, p = 0.055, Appendix 3.4.24) and small tillers ($F_{12, 24}$ = 1.18, p = 0.353, Appendix 3.4.25). There is no significant difference between the means in the grain filling percentage of either large tillers ($F_{12, 24}$ = 1.01, p = 0.473, Appendix 3.4.26) or small tillers ($F_{12, 24}$ = 0.85, p = 0.601, Appendix 3.4.27).

Table 3.7 The wheat cultivar Orator (2013/14) thousand grain weight (TGW) overall, TGW at position 1 to 2, and 3 to 5 for large and small tillers following INCYDE and TDZ-K treatment.

Treatment	Large TGW Overall (g)	Small TGW Overall (g)	Large TGW Pos 1-2 (g)	Small TGW Pos 1-2 (g)	Large TGW Pos 3-5 (g)	Small TGW Pos 3-5 (g)
Nil	40.0 ± 0.6	38.4 ± 0.6	45.4 ± 1.2	37.2 ± 2.4	34.5 ± 1.0	30.7 ± 2.2
DMSO Control (GS 39, 51, 61, 65)	43.3 ± 1.0	39.1 ± 3.5	44.2 ± 5.2	40.6 ± 3.1	36.7 ± 3.2	32.1 ± 4.4
DMSO Control (GS 61, 65, 65+13d)	41.8 ± 2.7	38.8 ± 0.7	44.5 ± 1.8	40.8 ± 1.1	34.8 ± 4.6	31.3 ± 1.7
INCYDE 10 µM (GS 65)	41.0 ± 1.0	40.5 ± 1.6	41.6 ± 1.8	39.4 ± 2.5	33.3 ± 1.6	30.1 ± 0.8
INCYDE 25 µM (GS 39, 51, 61, 65)	41.4 ± 2.2	33.3 ± 4.9	45.6 ± 3.4	36.1 ± 7.7	34.6 ± 2.6	24.7 ± 6.4
INCYDE 25 µM (GS 39)	43.4 ± 1.6	35.7 ± 0.3	49.6 ± 2.3	43.8 ± 1.0	36.6 ± 0.7	34.0 ± 2.6
INCYDE 25 µM (GS 51)	40.6 ± 0.7	34.9 ± 1.6	42.5 ± 1.8	36.7 ± 6.6	32.8 ± 4.2	31.4 ± 2.2
INCYDE 25 µM (GS 61)	38.4 ± 3.0	35.5 ± 4.7	37.3 ± 2.8	39.0 ± 3.8	30.1 ± 2.9	29.0 ± 1.9
INCYDE 25 µM (GS 65)	40.8 ± 1.3	39.7 ± 1.3	42.3 ± 2.2	41.2 ± 0.6	33.9 ± 1.9	33.9 ± 0.7
INCYDE 50 µM (GS 61)	42.2 ± 1.7	42.3 ± 1.6	47.6 ± 1.5	42.3 ± 2.1	36.5 ± 2.4	36.6 ± 1.8
INCYDE 50 µM (GS 65)	42.0 ± 0.8	36.0 ± 2.7	46.3 ± 1.2	39.8 ± 2.1	34.0 ± 1.7	32.0 ± 2.0
TDZ-K 10 µM (GS 61, 65, 65+13d)	42.9 ± 3.1	39.1 ± 1.7	46.7 ± 2.9	40.9 ± 4.7	37.6 ± 1.6	31.6 ± 3.4
TDZ-K 25 µM (GS 61, 65, 65+13d)	38.4 ± 0.7	35.9 ± 0.5	37.6 ± 0.8	36.7 ± 1.0	30.5 ± 2.6	32.7 ± 2.4
Treatment *F*-value ($F_{12, 24}$)[a]	0.96	1.18	2.19	0.42	0.88	0.99
Treatment *p*-value	0.511	0.347	**0.05**	0.939	0.575	0.483
Statistics	**Appendix 3.4.14**	**Appendix 3.4.15**	**Appendix 3.4.16**	**Appendix 3.4.17**	**Appendix 3.4.18**	**Appendix 3.4.19**
Analysis notes	ANOVA	ANOVA	ANOVA, Tukey HSD	ANOVA	ANOVA	ANOVA

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

For wheat cultivar Orator (2013/14), the overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates/plots (n = 3), within separate blocks, arranged in a randomised complete block design. Analyses were carried out at the University of Canterbury by taking ten whole plants from each plot (30 in total for each treatment level), dividing plants into large and small tillers, and acquiring 30 grains (90 in total for a treatment level) at different positions along the heads and florets as described in section **3.2.4.2** and **Figures 3.2** and **3.3**. The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **3.2.2**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA, Tukey HSD and residual plots for the ANOVA assumptions are provided in **Appendices 3.4.14** to **3.4.19** for each trait. *F*-values and *p*-values are provided for TGW overall, TGW at position 1 to 2, and 3 to 5 for the treatment factor. For more information on the experimental design and analyses see section **3.2.4**.

Table 3.8 The wheat cultivar Orator (2013/14) thousand grain weight (TGW) of the top third, middle third, bottom third and grain filling percentage for large and small tillers following INCYDE and TDZ-K treatment.

Treatment	Large TGW Top 1/3 (g)	Small TGW Top 1/3 (g)	Large TGW Middle 1/3 (g)	Small TGW Middle 1/3 (g)	Large TGW Bottom 1/3 (g)	Small TGW Bottom 1/3 (g)	Large Grain Filling (%)	Small Grain Filling (%)
Nil	32.7 ± 1.3	31.4 ± 2.6	38.4 ± 1.1	37.2 ± 1.4	45.2 ± 0.1	40.8 ± 2.7	59.1 ± 1.1	53.1 ± 0.8
DMSO Control (GS 39, 51, 61, 65)	35.6 ± 5.1	30.5 ± 1.3	44.3 ± 3.0	39.2 ± 3.9	48.4 ± 1.7	45.3 ± 3.2	57.3 ± 1.9	53.0 ± 3.8
DMSO Control (GS 61, 65, 65+13d)	34.8 ± 1.6	31.9 ± 1.9	43.3 ± 3.5	41.4 ± 1.3	47.2 ± 4.9	42.9 ± 1.0	58.9 ± 2.0	53.2 ± 3.9
INCYDE 10 µM (GS 65)	31.0 ± 1.3	32.5 ± 1.7	36.7 ± 1.6	39.2 ± 1.7	41.2 ± 2.3	41.4 ± 1.1	56.7 ± 1.3	48.6 ± 1.3
INCYDE 25 µM (GS 39, 51, 61, 65)	35.1 ± 4.1	26.0 ± 5.1	44.3 ± 2.1	32.5 ± 7.5	46.6 ± 1.3	37.3 ± 9.2	56.4 ± 1.1	53.5 ± 2.6
INCYDE 25 µM (GS 39)	37.9 ± 2.8	30.3 ± 1.8	49.8 ± 0.6	40.5 ± 1.7	49.8 ± 0.3	40.6 ± 2.1	56.8 ± 0.5	53.6 ± 1.1
INCYDE 25 µM (GS 51)	31.4 ± 4.9	27.0 ± 2.7	40.4 ± 2.1	36.7 ± 3.9	43.5 ± 2.0	39.3 ± 3.0	59.6 ± 0.9	51.2 ± 0.7
INCYDE 25 µM (GS 61)	31.1 ± 2.5	27.4 ± 3.6	39.1 ± 4.2	34.8 ± 2.4	40.8 ± 2.5	40.1 ± 4.4	61.7 ± 1.6	50.3 ± 3.2
INCYDE 25 µM (GS 65)	32.7 ± 2.0	31.9 ± 1.2	41.8 ± 1.3	43.1 ± 0.6	46.4 ± 0.4	44.0 ± 2.6	59.6 ± 1.1	53.6 ± 1.1
INCYDE 50 µM (GS 61)	36.9 ± 1.7	33.5 ± 2.8	45.7 ± 2.9	44.1 ± 1.1	46.1 ± 2.4	44.9 ± 2.8	57.3 ± 3.1	51.5 ± 3.7
INCYDE 50 µM (GS 65)	34.3 ± 1.5	29.5 ± 3.9	44.7 ± 3.1	41.1 ± 1.3	47.7 ± 2.4	45.0 ± 0.8	57.3 ± 2.8	51.5 ± 1.4
TDZ-K 10 µM (GS 61, 65, 65+13d)	36.8 ± 2.4	29.6 ± 2.0	45.9 ± 1.9	40.1 ± 3.7	49.4 ± 2.3	42.4 ± 0.9	60.5 ± 1.5	52.9 ± 2.6
TDZ-K 25 µM (GS 61, 65, 65+13d)	30.2 ± 0.2	32.4 ± 2.3	36.5 ± 2.0	37.5 ± 1.7	42.6 ± 1.5	42.5 ± 1.0	54.8 ± 3.0	46.1 ± 2.6
Treatment *F*-value ($F_{12, 24}$)[a]	0.91	0.69	2.44	1.26	2.14	1.18	1.01	0.85
Treatment *p*-value	0.554	0.744	**0.03**	0.303	0.055	0.353	0.473	0.601
Statistics	**Appendix 3.4.20**	**Appendix 3.4.21**	**Appendix 3.4.22**	**Appendix 3.4.23**	**Appendix 3.4.24**	**Appendix 3.4.25**	**Appendix 3.4.26**	**Appendix 3.4.27**
Analysis notes	ANOVA	ANOVA	ANOVA, Tukey HSD	ANOVA	ANOVA	ANOVA	Logit-transformed, ANOVA	Logit-transformed, ANOVA

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

For wheat cultivar Orator (2013/14), the overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates/plots (n = 3), within separate blocks, arranged in a randomised complete block design. Analyses were carried out at the University of Canterbury by taking ten whole plants from each plot (30 in total for each treatment level), dividing plants into large and small tillers, and acquiring 30 grains (90 in total for a treatment level) at different positions along the heads and florets as described in section **3.2.4.2** and **Figures 3.2** and **3.3**. The grain filling percentage was determined by counting the number of filled positions on five heads for each plot (15 in total for a treatment level). The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **3.2.2**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA, Tukey HSD and residual plots for the ANOVA assumptions are provided in **Appendices 3.4.20** to **3.4.27**. *F*-values and *p*-values are provided for the TGW top third, middle third, bottom third sections of heads and the grain filling percentage for the treatment factor. Grain filling percentage data was logit-transformed prior to ANOVA. For more information on the experimental design and analyses see section **3.2.4**.

An analysis of the progression of senescence (yellowing) of primary leaves was determined following infection of the wheat Orator trial with *Septoria tritici*. There is no statistically significant difference between the means of the proportion of leaf yellow (senescence) following infection with *Septoria tritici* ($F_{6, 12} = 1.09$, p = 0.421, Appendix 3.4.28).

Table 3.9 The proportion of yellow in primary leaves following *Septoria tritici* infection in wheat cultivar Orator (2013/14) following INCYDE and TDZ-K treatment.

Treatment	Proportion of yellow
DMSO Control	0.50 ± 0.03
NIL	0.40 ± 0.05
TDZ-K 10 µM	0.38 ± 0.06
TDZ-K 25 µM (GS 61, 65, 65+13d)	0.39 ± 0.02
INCYDE 10 µM (GS 65)	0.42 ± 0.02
INCYDE 25 µM (GS 39, 51, 61, 65)	0.47 ± 0.03
INCYDE 50 µM (GS 65)	0.47 ± 0.05
Treatment *F*-value ($F_{6, 12}$)[a]	1.09
Treatment *p*-value	0.421
Statistics	**Appendix 3.4.28**
Analysis notes	Logit-transformed, ANOVA

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

For wheat cultivar Orator (2013/14), the overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates/plots (n = 3), within separate blocks, arranged in a randomised complete block design. Analyses were carried out at the University of Canterbury, by taking primary leaves from nine different plants from each plot (27 in total for a treatment level) and determining the proportion of primary leaf that had yellow (senescence), as described in section **3.2.6**. The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **3.2.2**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Proportion data was logit-transformed prior to ANOVA and provided in **Appendix 3.4.28**. *F*-values and *p*-values are provided for the treatment factor.

3.3.4 Torch wheat yield, dry weight and growth

A second wheat field trial, carried out in the 2014/15 season with wheat cultivar Torch, focused on determining the effect of CPPU and TDZ-K. As INCYDE did not result in any significant change in growth or yield in the previous wheat Orator field trial, field work using INCYDE targeted at wheat was discontinued and instead INCYDE was applied to barley cultivar Quench. The reasoning for focusing on targeting anthesis and early senescence with a concentration range between 10 and 100 µM is described in section **3.1.2**. With the wheat cultivar Torch, there was no statistically significant difference between the means in the number of tillers (Poisson regression: p = 0.995, Appendix 3.5.7) (**Table 3.10**). ANOVA revealed that there were no statistically significant differences between the means in of the head length of large tillers ($F_{9, 18}$ = 1.48, p = 0.228, Appendix 3.5.1) and small tillers ($F_{9, 18}$ = 0.76, p = 0.655, Appendix 3.5.2), head DW of large tillers ($F_{9, 18}$ = 1.83, p = 0.131, Appendix 3.5.3) and small tillers ($F_{9, 18}$ = 0.64, p = 0.75, Appendix 3.5.4), stem length of large tillers ($F_{9, 18}$ = 0.64, p = 0.746, Appendix 3.5.5) and small tillers ($F_{9, 18}$ = 0.8, p = 0.62, Appendix 3.5.6), or in the means of the stem DW (**Table 3.11**) of large tillers ($F_{9, 18}$ = 1.87, p = 0.123, Appendix 3.5.8) and small tillers ($F_{9, 18}$ = 2.15, p = 0.08, Appendix 3.5.9).

With the TGW of large tillers (**Table 3.11**), the TGW of grain in plants treated with CPPU 30 µM (GS 61, 65) treatment is significantly smaller than that of the controls ($F_{9, 18}$ = 8.67, p < 0.001, *post hoc* Tukey HSD, confidence interval: 95%, Appendix 3.5.10). While ANOVA revealed that the *p*-value was < 0.05 for the treatment factor with the TGW overall in smaller tillers ($F_{9, 18}$ = 2.65, p = 0.037, Appendix 3.5.11), and the TGW of position 1 to 2 of large tillers ($F_{9, 18}$ = 8.87, p < 0.001, Appendix 3.5.12) and small tillers ($F_{9, 18}$ = 3.76, p = 0.008, Appendix 3.5.13), a *post hoc* pairwise comparisons of the means with Tukey HSD revealed that there was no significant difference between any treatment and both the controls.

Table 3.10 The wheat cultivar Torch (2014/15) tiller number, head length, head dry weight, stem length for large and small tillers following TDZ-K and CPPU treatment.

Treatment	Large Head Length (mm)	Small Head Length (mm)	Large Head DW (g)	Small Head DW (g)	Large Stem Length (mm)	Small Stem Length (mm)	Tiller no.
Nil	84.5 ± 0.6	80.4 ± 0.7	3.2 ± 0.1	2.3 ± 0.2	642.6 ± 3.1	622.7 ± 44.3	3.1 ± 0.2
DMSO Control (GS 51, 61, 65, 65+15d)	83.6 ± 2.3	76.8 ± 1.2	3.3 ± 0.2	2.3 ± 0.1	645.9 ± 8.6	608.8 ± 23.0	3.1 ± 0.5
TDZ-K 10 µM (GS 61, 65, 65+15d)	85.3 ± 1.1	80.9 ± 2.2	3.4 ± 0.1	2.5 ± 0.1	641.2 ± 29.9	660.0 ± 7.4	4.0 ± 0.6
TDZ-K 50 µM (GS 61, 65, 65+15d)	85.6 ± 0.9	77.8 ± 1.7	3.1 ± 0.1	2.4 ± 0.1	654.3 ± 26.3	618.9 ± 24.2	2.8 ± 0.3
CPPU 10 µM (GS 61, 65)	88.1 ± 0.8	80.5 ± 3.5	3.1 ± 0.2	2.1 ± 0.3	678.3 ± 41.4	607.4 ± 21.2	3.8 ± 0.7
CPPU 30 µM (GS 61, 65)	84.9 ± 1.3	78.7 ± 2.1	2.9 ± 0.2	2.3 ± 0.1	668.8 ± 11.4	640.9 ± 18.4	3.7 ± 0.2
CPPU 100 µM (GS 61, 65)	86.1 ± 1.0	79.7 ± 1.7	3.0 ± 0.1	2.2 ± 0.2	680.0 ± 9.1	654.7 ± 22.2	2.7 ± 0.4
CPPU 10 µM (GS 51, 65)	86.9 ± 2.6	77.6 ± 0.7	3.2 ± 0.3	2.3 ± 0.1	671.7 ± 17.8	636.4 ± 10.3	3.7 ± 0.5
CPPU 30 µM (GS 51, 65)	86.4 ± 1.6	76.8 ± 0.8	3.0 ± 0.2	2.3 ± 0.1	656.7 ± 21.7	654.9 ± 34.4	3.2 ± 0.3
CPPU 100 µM (GS 51, 65)	88.7 ± 0.4	79.6 ± 0.4	3.6 ± 0.1	2.4 ± 0.1	683.2 ± 15.2	586.6 ± 30.2	3.8 ± 0.8
Treatment *F*-value ($F_{9, 18}$)[a]	1.48	0.76	1.83	0.64	0.64	0.8	
Treatment *p*-value	0.228	0.655	0.131	0.75	0.746	0.62	0.995
Statistics	**Appendix 3.5.1**	**Appendix 3.5.2**	**Appendix 3.5.3**	**Appendix 3.5.4**	**Appendix 3.5.5**	**Appendix 3.5.6**	**Appendix 3.5.7**
Analysis notes	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA	Poisson regression

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

For wheat cultivar Torch (2014/15), the overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates/plots (n = 3), within separate blocks, arranged in a randomised complete block design. Analyses were carried out at the University of Canterbury by taking ten whole plants from each plot (30 in total for each treatment level), dividing plants into large and small tillers as described in section **3.2.4.2** and **Figure 3.2**. The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **3.2.2**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA, Poisson regression and residual plots for the ANOVA assumptions are provided in **Appendices 3.5.1** to **3.5.7**. *F*-values and *p*-values are provided for non-count data (head length, head dry weight and stem length) for the treatment factor. For count data (tiller number), a *p*-value is provided for the treatment factor of a Poisson regression. For more information on the experimental design and analyses see section **3.2.4**.

Table 3.11 The wheat cultivar Torch (2014/15) stem dry weight, thousand grain weight overall and at positions 1 to 2 for large and small tillers following TDZ-K and CPPU treatment.

Treatment	Large Stem DW (g)	Small Stem DW (g)	Large TGW Overall (g)	Small TGW Overall (g)	Large TGW Pos 1-2 (g)	Small TGW Pos 1-2 (g)
Nil	1.5 ± 0.1	1.5 ± 0.1	48.4 ± 0.7	43.7 ± 0.6	49.0 ± 0.8	45.5 ± 1.1
DMSO Control (GS 51, 61, 65, 65+15d)	1.6 ± 0.1	1.2 ± 0.04	51.5 ± 0.4	46.0 ± 1.9	53.6 ± 0.6	48.0 ± 0.9
TDZ-K 10 µM (GS 61, 65, 65+15d)	1.8 ± 0.1	1.4 ± 0.1	50.9 ± 0.3	45.4 ± 0.4	52.7 ± 0.9	46.4 ± 0.8
TDZ-K 50 µM (GS 61, 65, 65+15d)	1.7 ± 0.1	1.3 ± 0.1	48.8 ± 0.8	44.8 ± 0.5	50.1 ± 0.1	47.4 ± 0.3
CPPU 10 µM (GS 61, 65)	1.7 ± 0.1	1.3 ± 0.1	47.3 ± 1.2	41.0 ± 0.5	48.3 ± 1.6	41.6 ± 0.5
CPPU 30 µM (GS 61, 65)	1.7 ± 0.1	1.2 ± 0.02	**44.3 ± 0.4***	42.9 ± 1.4	45.0 ± 0.7	45.6 ± 1.4
CPPU 100 µM (GS 61, 65)	1.8 ± 0.04	1.3 ± 0.1	47.1 ± 0.8	45.8 ± 1.8	48.9 ± 0.9	47.3 ± 0.3
CPPU 10 µM (GS 51, 65)	1.8 ± 0.05	1.4 ± 0.1	49.3 ± 0.9	45.7 ± 1.6	50.0 ± 1.0	48.3 ± 1.5
CPPU 30 µM (GS 51, 65)	1.6 ± 0.1	1.5 ± 0.1	46.3 ± 0.7	43.4 ± 1.3	48.4 ± 0.7	45.4 ± 0.4
CPPU 100 µM (GS 51, 65)	1.9 ± 0.1	1.3 ± 0.1	48.6 ± 0.2	47.9 ± 1.2	49.7 ± 0.6	47.8 ± 1.5
Treatment *F*-value ($F_{9, 18}$)[a]	1.87	2.15	8.67	2.65	8.87	3.76
Treatment *p*-value	0.123	0.08	**< 0.001**	**0.037**	**< 0.001**	**0.008**
Statistics	**Appendix 3.5.8**	**Appendix 3.5.9**	**Appendix 3.5.10**	**Appendix 3.5.11**	**Appendix 3.5.12**	**Appendix 3.5.13**
Analysis notes	ANOVA	ANOVA	ANOVA, Tukey HSD, Levene's *p*-value: 0.958	ANOVA, Tukey HSD	ANOVA, Tukey HSD	ANOVA, Tukey HSD

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

* Indicates a statistically significant difference (Tukey HSD, confidence interval: 95%) for a treatment compared to both the DMSO control and the 'Nil' control.

For wheat cultivar Torch (2014/15), the overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates/plots (n = 3), within separate blocks, arranged in a randomised complete block design. Analyses were carried out at the University of Canterbury by taking ten whole plants from each plot (30 in total for each treatment level), dividing plants into large and small tillers, and acquiring 30 grains (90 in total for a treatment level) at different positions along the heads and florets as described in section **3.2.4.2** and **Figures 3.2** and **3.3**. The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **3.2.2**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA, Tukey HSD and residual plots for the ANOVA assumptions are provided in **Appendices 3.5.8** to **3.5.13**. *F*-values and *p*-values are provided for the stem dry weight, thousand grain weight overall and at positions 1 to 2 for the treatment factor. For more information on the experimental design and analyses see section **3.2.4**.

3.3.5 Quench barley yield, dry weight and growth

A barley field trial, carried out in the 2014/15 season with wheat cultivar Quench, focused on determining the effect of INCYDE on barley. As INCYDE did not affect growth or yield in wheat, its affect on barley was determined. The reasoning for focusing on targeting anthesis and early senescence with a concentration range between 10 and 50 µM is described in section **3.1.2**. With barley cultivar Quench, there was a statistically significant decrease in the TGW in small tillers following INCYDE 50 µM (GS 65) treatment compared to both controls ($F_{9, 18} = 7.13$, $p < 0.001$, *post hoc* Tukey HSD, confidence interval: 95%, Appendix 3.6.11) (**Table 3.13**).

While ANOVA revealed *p*-values < 0.05 for the treatment factor, including for the small tiller head length ($F_{9, 18} = 3.88$, $p = 0.007$, Appendix 3.6.2) (**Table 3.12**), small tiller head dry weight ($F_{9, 18} = 6.66$, $p < 0.001$, Appendix 3.6.4), large tiller stem length ($F_{9, 18} = 2.84$, $p = 0.028$, Appendix 3.6.6), and the large tiller TGW ($F_{9, 18} = 6.31$, $p < 0.001$, Appendix 3.6.10) (**Table 3.13**), *post hoc* Tukey HSD tests showed that there was no statistically significant difference between the treatments and control groups. ANOVA showed that there are no statistically significant differences between treatments and controls for the large tiller head length ($F_{9, 18} = 2.0$, $p = 0.101$, Appendix 3.6.1), large tiller head DW ($F_{9, 18} = 1.33$, $p = 0.29$, Appendix 3.6.3), small tiller stem length ($F_{9, 18} = 1.36$, $p = 0.278$, Appendix 3.6.7) or for the stem DW of large tillers ($F_{9, 18} = 1.29$, $p = 0.306$, Appendix 3.6.8) and small tillers ($F_{9, 18} = 2.27$, $p = 0.066$, Appendix 3.6.9).

Table 3.12 The barley cultivar Quench (2014/15) for tiller number, head length and head dry weight for large and small tillers following INCYDE treatment.

Treatment	Large Head Length (mm)	Small Head Length (mm)	Large Head DW (g)	Small Head DW (g)	Tiller no.
Nil	82.4 ± 0.9	52.8 ± 4.5	1.4 ± 0.04	0.7 ± 0.04	6.8 ± 0.6
DMSO Control (GS 51, 61, 65, 65+15d)	83.5 ± 1.1	62.8 ± 1.6	1.4 ± 0.05	0.9 ± 0.02	5.8 ± 0.2
INCYDE 10 µM (GS 65)	78.7 ± 1.0	54.2 ± 3.2	1.4 ± 0.07	0.7 ± 0.07	5.3 ± 0.6
INCYDE 25 µM (GS 39, 51, 61, 65)	79.9 ± 1.9	61.7 ± 3.3	1.3 ± 0.06	0.9 ± 0.02	6.9 ± 0.5
INCYDE 25 µM (GS 39)	78.4 ± 3.9	54.8 ± 3.6	1.3 ± 0.05	0.8 ± 0.07	5.6 ± 0.6
INCYDE 25 µM (GS 51)	77.2 ± 2.1	58.4 ± 2.3	1.4 ± 0.10	0.9 ± 0.05	5.9 ± 0.6
INCYDE 25 µM (GS 61)	79.8 ± 1.5	57.1 ± 1.1	1.4 ± 0.07	0.8 ± 0.08	4.7 ± 0.4
INCYDE 25 µM (GS 65)	72.2 ± 3.0	67.5 ± 2.7	1.2 ± 0.09	1.1 ± 0.03	5.8 ± 0.6
INCYDE 50 µM (GS 61)	78.5 ± 1.0	55.9 ± 1.9	1.4 ± 0.05	0.7 ± 0.06	5.4 ± 0.4
INCYDE 50 µM (GS 65)	77.3 ± 2.0	54.9 ± 1.5	1.3 ± 0.07	0.7 ± 0.03	5.6 ± 0.7
Treatment *F*-value ($F_{9, 18}$)[a]	2.0	3.88	1.33	6.66	
Treatment *p*-value	0.101	**0.007**	0.29	**<0.001**	0.991
Statistics	**Appendix 3.6.1**	**Appendix 3.6.2**	**Appendix 3.6.3**	**Appendix 3.6.4**	**Appendix 3.6.5**
Analysis notes	ANOVA	ANOVA, Tukey HSD	ANOVA	ANOVA, Tukey HSD	Poisson regression

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

For barley cultivar Quench (2014/15), the overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates/plots (n = 3), within separate blocks, arranged in a randomised complete block design. Analyses were carried out at the University of Canterbury by taking ten whole plants from each plot (30 in total for each treatment level), dividing plants into large and small tillers as described in section **3.2.4.2** and **Figure 3.2**. The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **3.2.2**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA, Tukey HSD, Poisson regression and residual plots for the ANOVA assumptions are provided in **Appendices 3.6.1** to **3.6.5**. *F*-values and *p*-values are provided for non-count data (head length and head dry weight) for the treatment factor. For count data (tiller number), a *p*-value is provided for the treatment factor of a Poisson regression. For more information on the experimental design and analyses see section **3.2.4**.

Table 3.13 The barley cultivar Quench (2014/15) for stem length, stem dry weight and thousand grain weight overall for large and small tillers following INCYDE treatment.

Treatment	Large Stem Length (mm)	Small Stem Length (mm)	Large Stem DW (g)	Small Stem DW (g)	Large TGW (g)	Small TGW (g)
Nil	690.1 ± 11.6	641.9 ± 15.9	0.8 ± 0.02	0.5 ± 0.02	55.0 ± 0.2	48.2 ± 1.3
DMSO Control (GS 51, 61, 65, 65+15d)	659.6 ± 21.3	632.1 ± 33.3	0.7 ± 0.02	0.5 ± 0.04	52.4 ± 0.8	49.6 ± 0.8
INCYDE 10 µM (GS 65)	691.6 ± 6.9	639.0 ± 36.4	0.7 ± 0.05	0.6 ± 0.03	55.0 ± 0.5	47.4 ± 0.7
INCYDE 25 µM (GS 39, 51, 61, 65)	627.5 ± 12.3	614.2 ± 5.6	0.7 ± 0.02	0.5 ± 0.01	49.3 ± 1.3	44.8 ± 1.3
INCYDE 25 µM (GS 39)	684.2 ± 15.3	677.7 ± 18.2	0.7 ± 0.04	0.6 ± 0.01	53.5 ± 0.8	49.6 ± 0.7
INCYDE 25 µM (GS 51)	664.5 ± 20.4	653.5 ± 13.6	0.7 ± 0.02	0.5 ± 0.05	53.0 ± 1.1	49.5 ± 0.3
INCYDE 25 µM (GS 61)	688.5 ± 12.2	664.0 ± 16.7	0.7 ± 0.05	0.6 ± 0.04	51.3 ± 0.2	51.0 ± 1.0
INCYDE 25 µM (GS 65)	674.1 ± 7.8	687.1 ± 4.9	0.6 ± 0.05	0.7 ± 0.03	49.7 ± 0.8	49.5 ± 1.3
INCYDE 50 µM (GS 61)	674.9 ± 7.4	633.8 ± 43.7	0.7 ± 0.03	0.5 ± 0.06	50.8 ± 1.4	46.4 ± 0.6
INCYDE 50 µM (GS 65)	618.3 ± 24.5	586.7 ± 38.0	0.7 ± 0.05	0.5 ± 0.06	54.9 ± 0.8	**42.9 ± 0.7***
Treatment *F*-value ($F_{9, 18}$)[a]	2.84	1.36	1.29	2.27	6.31	7.13
Treatment *p*-value	**0.028**	0.278	0.306	0.066	**< 0.001**	**< 0.001**
Statistics	**Appendix 3.6.6**	**Appendix 3.6.7**	**Appendix 3.6.8**	**Appendix 3.6.9**	**Appendix 3.6.10**	**Appendix 3.6.11**
Analysis notes	ANOVA, Tukey HSD	ANOVA	ANOVA	ANOVA	ANOVA, Tukey HSD	ANOVA, Tukey HSD, Levene's *p*-value: 0.967

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

* Indicates a statistically significant difference (Tukey HSD, confidence interval: 95%) for a treatment compared to both the DMSO control and the 'Nil' control.

For barley cultivar Quench (2014/15), the overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates/plots (n = 3), within separate blocks, arranged in a randomised complete block design. Analyses were carried out at the University of Canterbury by taking ten whole plants from each plot (30 in total for each treatment level), dividing plants into large and small tillers, and acquiring 30 grains (90 in total for a treatment level) at different positions along the heads as described in section **3.2.4.2** and **Figures 3.2** and **3.3**. The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **3.2.2**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA, Tukey HSD and residual plots for the ANOVA assumptions are provided in **Appendices 3.6.6** to **3.6.11**. *F*-values and *p*-values are provided for stem length, stem dry weight and TGW overall for the treatment factor. For more information on the experimental design and analyses see section **3.2.4**.

3.3.6 The effect of treatments on chlorophyll content in wheat

The effect of each PGR was determined on wheat and barley by using the 2014/15 wheat and barley field trials. Only these trials were selected for chlorophyll analysis as the Orator trial was infected with *Septoria*. For the wheat cultivar Torch, the chlorophyll content of treatments was compared to control groups at different time points (0, 2 and 4 weeks after treatment) (**Table 3.14**). For the primary leaf of wheat, an ANOVA showed a significant effect for the time factor ($F_{2, 24} = 124.22$, $p < 0.001$, Appendix 3.7.2), the treatment ($F_{2, 24} = 9.26$, $p = 0.001$) and an interaction between the time and treatment ($F_{4, 24} = 4.15$, $p = 0.011$). The interaction plot showed a disordinal interaction, where the TDZ-K treatment crossed over the control between 2 and 4 weeks after treatment. In contrast, the control and CPPU lines are parallel at every time point, indicating no interaction effect when comparing the control and CPPU. A *post hoc* Tukey HSD test (confidence interval: 95%) (Appendix 3.7.2) showed that there was significant loss (or increase in rate of loss) of chlorophyll following TDZ-K treatment at 2 weeks after treatments compared to the control at 2 weeks. The *post hoc* Tukey HSD test also showed that plants treated with CPPU had significantly more chlorophyll retention compared to the control plants.

In the flag leaves of wheat (**Table 3.14**), there was only a significant effect of time on the chlorophyll content ($F_{2, 24} = 75.08$, $p < 0.001$, Appendix 3.7.1), with a statistically significant loss of chlorophyll in plants from 2 to 4 weeks after treatment (*post hoc* Tukey HSD test, confidence interval: 95%). Analyses of variance of the secondary leaves of wheat indicated a p-value ≤ 0.05 for both the time factor ($F_{1, 15} = 7.56$, $p = 0.015$) and the treatment factor ($F_{2, 15} = 6.30$, $p = 0.010$) and a *post hoc* Tukey HSD showed a significant loss of chlorophyll following TDZ-K treatment compared to the control. A *post hoc* Tukey HSD test (Appendix 3.7.3) revealed a significant loss of chlorophyll at 2 weeks compared to week 0 after the treatment period. There was no statistically significant difference at a specific time point in the content of chlorophyll in either of the treatments compared to the control for the same time point.

Table 3.14 The effect of TDZ-K and CPPU on the content of chlorophyll in flag, primary and secondary leaves of wheat cultivar Torch (2014/15) at 0, 2 and 4 weeks following the end of the last treatment (15 days after GS 65). TDZ-K was applied at 50 µM (GS 61, 65, 65+15d) and CPPU at 100 µM (GS 51, 65).

		Chlorophyll content (mg/g) weeks after last treatment			
Leaf sample	Treatments	Week 0	2 weeks	4 weeks	Analysis notes
Flag	Control	4.5 ± 0.4	4.8 ± 0.1	0.9 ± 0.1	ANOVA, Tukey HSD
	TDZ-K 50 µM	5.3 ± 0.6	4.2 ± 0.4	1.0 ± 0.4	
	CPPU 100 µM	4.7 ± 0.3	5.1 ± 0.3	1.8 ± 0.5	
	ANOVA	Time ($F_{2, 24}$)[a]	Treatment ($F_{2, 24}$)[a]	Time*Treatment ($F_{4, 24}$)[a]	
	F-value	75.08	1.14	1.25	
	p-value	< **0.001**	0.335	0.316	
	Statistics	**Appendix 3.7.1**			
Primary	Control	5.0 ± 0.4	4.8 ± 0.2	0.3 ± 0.1	ANOVA, Tukey HSD, interaction plot
	TDZ-K 50 µM	5.0 ± 0.4	**2.9 ± 0.2***	0.9 ± 0.1	
	CPPU 100 µM**	5.7 ± 0.5	5.5 ± 0.6	1.4 ± 0.5	
	ANOVA	Time ($F_{2, 24}$)[a]	Treatment ($F_{2, 24}$)[a]	Time*Treatment ($F_{4, 24}$)[a]	
	F-value	124.22	9.26	4.15	
	p-value	< **0.001**	**0.001**	**0.011**	
	Statistics	**Appendix 3.7.2**			
Secondary	Control	4.0 ± 0.3	3.8 ± 0.4		ANOVA, Tukey HSD
	TDZ-K 50 µM**	3.6 ± 0.3	1.9 ± 0.2		
	CPPU 100 µM	4.9 ± 0.5	3.7 ± 0.8		
	ANOVA	Time ($F_{1, 15}$)[a]	Treatment ($F_{2, 15}$)[a]	Time*Treatment ($F_{2, 15}$)[a]	
	F-value	7.56	6.30	1.34	
	p-value	**0.015**	**0.010**	0.292	
	Statistics	**Appendix 3.7.3**			

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

* Indicates a statistically significant difference (*post hoc* Tukey HSD, confidence interval: 95%) for a treatment compared to the control at a specific time point.

** Indicates a statistically significant difference (*post hoc* Tukey HSD, confidence interval: 95%) of the treatment compared to the control.

The values provided are the overall mean chlorophyll content (mg/g) for four independent replicates/plots (n = 4) of wheat cultivar Torch (2014/15), within separate blocks, arranged in a randomised complete block design. The weeks are given as the number of weeks from the last treatment application (15 days after GS 65). For each time point, from a single plot a subsample of ten plants (30 in total for a treatment level) were acquired. Analyses were carried out at the University of Canterbury by taking flag, primary and secondary leaves from the large/main stems and analysing the leaves according to Evans *et al*. (2012). More details are provided in section **3.2.4.3**. ANOVA analyses were carried out by partitioning for the treatment factor, time factor, block factor and the time*treatment interaction. *F*-values and *p*-values are provided for the ANOVA analyses, which are described in **Appendices 3.7.1** to **3.7.3**. A *post hoc* Tukey HSD (confidence interval: 95%) was carried out when $p \leq 0.05$ for any of the factors of interest (treatment, time or interaction). An interaction plot was described in the Appendix when ANOVA revealed an interaction effect ($p \leq 0.05$).

3.3.7 The effect of treatments on chlorophyll content in barley

In the barley cultivar Quench, there are only statistically significant differences in the content of chlorophyll at different time points, with the time factor *p*-value ≤ 0.05 (**Table 3.15**). In flag leaves ($F_{2, 24}$ = 212.73, p < 0.001, Appendix. 3.8.1), there is a statistically significant increase in the content of chlorophyll from 0 to 2.5 weeks after treatment, and a significant loss of chlorophyll from 2.5 to 5 weeks (*post hoc* Tukey HSD, confidence interval: 95%). In primary leaves, there is a statistically significant loss of chlorophyll from 2.5 to 5 weeks after treatment ($F_{2, 24}$ = 72.21, p < 0.001, *post hoc* Tukey HSD, confidence interval: 95%, Appendix 3.8.2). For secondary leaves, there is a significant loss of chlorophyll of plants from 0 to 2.5 weeks after treatment ($F_{2, 24}$ = 53.27, p < 0.001, *post hoc* Tukey HSD, p ≤ 0.05, Appendix 3.8.3), while for tertiary leaves, there was a statistically significant loss in the chlorophyll content from 0 to 2.5 weeks after treatment ($F_{2, 24}$ = 24.04, p < 0.001, *post hoc* Tukey HSD, confidence interval: 95%, Appendix 3.8.4).

Table 3.15 The effect of INCYDE on the content of chlorophyll in flag, primary, secondary and tertiary leaves of barley cultivar Quench (2014/15) at 0, 2.5 and 5 weeks following the end of the last treatment (GS 65). INCYDE was applied at 25 µM (GS 39, 51, 61, 65) and 50 µM (GS 61).

		Chlorophyll content (mg/g) weeks after last treatment			
Leaf sample	Treatments	Week 0	2.5 weeks	5 weeks	Analysis notes
Flag	Control	7.1 ± 1.0	10.2 ± 1.0	0.3 ± 0.03	ANOVA, Tukey HSD
	INCYDE 25 µM	7.2 ± 0.8	10.2 ± 0.7	0.8 ± 0.2	
	INCYDE 50 µM	7.0 ± 0.4	9.6 ± 0.5	0.3 ± 0.05	
	ANOVA	Time ($F_{2, 24}$)[a]	Treatment ($F_{2, 24}$)[a]	Time*Treatment ($F_{4, 24}$)[a]	
	F-value	212.73	0.31	0.11	
	p-value	< **0.001**	0.738	0.979	
	Statistics	**Appendix 3.8.1**			
Primary	Control	5.7 ± 0.5	7.4 ± 1.5	0.4 ± 0.1	ANOVA, Tukey HSD
	INCYDE 25 µM	6.6 ± 0.7	6.9 ± 1.2	0.4 ± 0.1	
	INCYDE 50 µM	6.1 ± 0.7	6.1 ± 0.7	0.4 ± 0.1	
	ANOVA	Time ($F_{2, 24}$)[a]	Treatment ($F_{2, 24}$)[a]	Time*Treatment ($F_{4, 24}$)[a]	
	F-value	72.21	0.26	0.48	
	p-value	< **0.001**	0.770	0.750	
	Statistics	**Appendix 3.8.2**			
Secondary	Control	3.8 ± 0.2	2.5 ± 0.4		ANOVA, Tukey HSD
	INCYDE 25 µM	3.7 ± 0.2	1.6 ± 0.3		
	INCYDE 50 µM	3.4 ± 0.3	2.4 ± 0.4		
	ANOVA	Time ($F_{1, 15}$)[a]	Treatment ($F_{2, 15}$)[a]	Time*Treatment ($F_{2, 15}$)[a]	
	F-value	53.27	1.69	2.66	
	p-value	< **0.001**	0.218	0.103	
	Statistics	**Appendix 3.8.3**			
Tertiary	Control	2.5 ± 0.1	0.8 ± 0.2		ANOVA, Tukey HSD
	INCYDE 25 µM	2.9 ± 0.2	0.3 ± 0.1		
	INCYDE 50 µM	2.6 ± 0.3	2.2 ± 0.9		
	ANOVA	Time ($F_{1, 15}$)[a]	Treatment ($F_{2, 15}$)[a]	Time*Treatment ($F_{2, 15}$)[a]	
	F-value	24.04	2.58	3.53	
	p-value	< **0.001**	0.109	0.055	
	Statistics	**Appendix 3.8.4**			

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

The values provided are the overall mean chlorophyll content (mg/g) for four independent replicates/plots (n = 4) of barley cultivar Quench (2014/15), within separate blocks, arranged in a randomised complete block design. The weeks are given as the number of weeks from the last treatment application (GS 65). For each time point, from a single plot a subsample of ten plants (30 in total for a treatment level) were acquired. Analyses were carried out at the University of Canterbury by taking flag, primary, secondary and tertiary leaves from the large/main stems and analysing the leaves according to Evans *et al*. (2012). More details are provided in section **3.2.4.3**. ANOVA analyses were carried out by partitioning for the treatment factor, time factor, block factor and the time*treatment interaction. *F*-values and *p*-values are provided for the ANOVA analyses, which are described in **Appendices 3.87.1** to **3.8.4**. A *post hoc* Tukey HSD (confidence interval: 95%) was carried out when $p \leq 0.05$ for any of the factors of interest (treatment, time or interaction).

3.4 Discussion

3.4.1 Yield

The absence of any difference in yield between treatments and controls in the New Zealand Grainlab results for the wheat (**Tables 3.2** and **3.3**) and barley trials (**Table 3.4**) indicates that there are no immediately obvious effects of PGR treatments on the yield. Detailed hand analyses carried out with dissected heads, however, showed that some changes in TGW were in fact evident (**Tables 3.11** and **3.13**).

Other than a statistically significant decrease in the TGW overall in large tillers of wheat cultivar Torch following CPPU 30 µM (GS 61, 65) (**Table 3.11**), and a decrease in the TGW in small tillers following INCYDE 50 µM (GS 65) treatment in barley cultivar Quench (**Table 3.13**), there is little evidence of a change in growth or yield for either Torch (**Table 3.10** and **3.11**) or Quench (**Table 3.12** and **3.13**). The fact that changes in yield occurred only when these compounds were applied at specific concentrations (INCYDE at 50 µM and CPPU at 30 µM) at specific growth stages indicates the specificity of the effect, and the importance of both the concentration and growth stage targeting for inducing effects.

There are several possible reasons for the lack of change in yield (and even decrease) that was observed in yield when targeting anthesis. The elevated levels of cytokinin post-anthesis is known to be transient in wheat (Jameson *et al*., 1982; Banowetz *et al*., 1999b) and barley (Powell *et al*., 2013) in developing grains, and an upregulation of the expression of both *TaIPT* and *TaCKX* occurs transiently post-anthesis (Song *et al*., 2012). Despite the attempts to ensure that treatments were applied at anthesis (and the two applications made at GS 61 and 65 for some treatments) as described in section **3.2.2**, it is very possible this transient post-anthesis accumulation of cytokinin and increase in CKX activity (following expression) was missed by CKX-targeting INCYDE and CPPU in many plants (Bilyeu *et al*., 2001; Zatloukal *et al*., 2008), and as a consequence, endogenous cytokinin levels were not enhanced during this critical period of grain development.

Targeting anthesis might not have been the best approach to application. Recent communication with collaborators suggested that targeting earlier growth stages of development (before anthesis) in wheat and barley induced a clearer response (personal communication, February 24, 2017). There is other evidence to suggest that earlier growth stages might be better targets in future. Gene expression

studies have shown that there was a strong expression of genes associated with cytokinin regulation during inflorescence (panicle) development in rice (Yamburenko *et al*., 2017), suggesting that the developing inflorescence was a suitable PGR target. In rice, Ashikari *et al*. (2005) identified inflorescence-expressed *OsCKX2* as key to determining yield in rice, with an enhancement of yield evident in loss-of-function *Osckx2* plants and transgenic antisense targeting *OsCKX2*. However, it is noteworthy to point that the earliest growth stage targeted in these field trials was at the end of elongation (GS 39) targeted by INCYDE in wheat cultivar Orator (2013/14) and barley cultivar Quench (2014/15), and targeting wheat or barley at this growth stage did not result in any statistically significant difference in the yield in comparison to the control groups (**Tables 3.5**, **3.6**, **3.7**, **3.8**, **3.12** and **3.13**).

Additionally, difficulties with applying cytokinins or PGRs in the field are well-documented (Jameson and Song, 2016, and references therein). Replicating findings from controlled experiments has been met with challenges (Nagel *et al*., 2001), including controlling for the effect of the environment. The lack of any consistent yield enhancement might also in part be due to the complex and pleiotropic effects of cytokinin (Jameson and Song, 2016; Koprna *et al*., 2016), which make it difficult to predict the effect of applying PGRs to the whole plant. Cytokinin needs to be precisely and moderately increased in order to have beneficial effects on growth and/or yield (Guo and Gan, 2014, and references therein; Jameson and Song, 2016). Cytokinin needs to be targeted to increase the sink strength, at the cost of other sinks, of the head and developing grains.

The application of PGRs chlormequat chloride and Moddus to the wheat field trials (Appendix 3.1) might also have affected the efficacy of the PGRs. These compounds inhibit the biosynthesis of gibberellin, which would affect gibberellin cross-talk with cytokinin (Weiss and Ori, 2007), and these PGRs would reduce the resources used in stem elongation (reducing the strength of this competing sink), further optimising the trial, leaving little room for yield enhancement via PGR application.

Treatments were applied as a foliar spray using Yates Sprayfix at 0.5%. Yates Sprayfix might not have been the optimal surfactant for uptake of PGRs into the plants, which would limit the capacity of INCYDE and CPPU to increase cytokinin levels via inhibition of CKX. Effective methods for uptake have included injection which have resulted in increases in yield, including following BA injection into the shoot ear of wheat during anthesis (Sivakumar *et al*., 2001), and in maize during pollination (Dietrich *et al*., 1995). These responses indicate that cytokinin is limiting to seed yield but this is totally impractical. This indicates that the lack of yield enhancement and response might be because the method of spraying, and/or due to the surfactant used, which might have limited the uptake of PGR. However, the observations of a reduction in yield following INCYDE and CPPU suggest that these compounds likely got in to the plants.

An enhancement of cytokinin following application of INCYDE and CPPU, potentially resulted in increases in cytokinin degrading CKX activity and/or expression, an observation which has been made with other cereals following cytokinin application (Hirose *et al*., 2008; Vyroubalová *et al*., 2009). This increase in degradation might be one explanation for the reduction in yield observed following one INCYDE and CPPU treatment group targeted at anthesis, and multiple applications might have been required to overcome this feedback response.

3.4.2 Leaf senescence and the effect of *Septoria tritici*

Despite the reputed senescence inhibiting properties of TDZ-K with wheat leaves in detached senescence assays, applied between 0.1 and 100 µM (United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript), a more rapid decline in chlorophyll in the primary leaves was measured following TDZ-K treatment compared to the control at two weeks following treatment (**Table 3.14**). A more rapid decrease in chlorophyll content would reduce the time that leaves were able to photosynthesise, leading to a reduction in photoassimilates that would be translocated to the developing grains, translating into a reduction in the yield. However, in this case there remains no corresponding decline in yield in either wheat or barley following TDZ-K treatment.

There are a number of possibilities why this delay in senescence was not observed. Uptake of TDZ-K might have been limited by the method of uptake, the treatments may have, by some mechanism, increased endogenous cytokinin content, and induced a feedback response which might have had the effect of reducing endogenous cytokinin, and given that a reduction in cytokinin correlates with a loss

of chlorophyll (Buchanan-Wollaston, 1997; Noodén *et al*., 1997), this might help explain the reduction in chlorophyll compared to the control.

There was a greater retention of chlorophyll in primary leaves of wheat of plants treated with CPPU (**Table 3.14**). This observed retention could corresponded to the decline in yield following CPPU application in wheat (**Table 3.11**). Control over senescence is complex (Fischer, 2012), but there is evidence of the yield in rice decreasing in plants with slow senescing traits (Jiang *et al*., 2004; Rubia *et al*., 2014, and references therein), indicating that a delay in senescence could retard the remobilisation of photosynthates leading to reduced grain filling (Zhang *et al*., 1998; Rubia *et al*., 2014).

It was clear that PGRs had an effect on the chlorophyll content on wheat, and this effect appeared over the duration of senescence with CPPU treatment, but only at a specific time point (2 weeks) following TDZ-K treatment. This indicates that a potential feedback response to TDZ-K induced chlorophyll loss that might have occurred after 2 weeks.

When comparing the proportion of yellow (senescence) on primary wheat leaves following infection with *Septoria tritici*, there was no difference when treatments were compared to both controls (**Table 3.9**). This suggests that neither INCYDE nor TDZ-K conveyed resistance to this fungi. Further experiments assessing additional time points and other leaves (flag, secondary and tertiary) would be required to ascertain this.

3.4.3 Summary

It is clear that most of the treatments made in the field had no statistically significant effect on yield or growth of barley or wheat using high yielding cultivars, optimised fertiliser application and applied fungicides and PGRs. This indicated the difficulty of conducting field trials to measure the efficacy of INCYDE, TDZ-K and CPPU, and the difficulty in repeating the yield enhancement observed in the growth room experiments with RCBr (Chapter 2).

Of the changes in yield that were observed, the changes showed only a decrease in yield when anthesis was targeted with specific concentrations of INCYDE or CPPU. Several reasons were suggested for this including missing the transient increase in CKX activity, from an increase in *CKX* expression in wheat (Song *et al*., 2012), the activation of a feedback system which might reduce

endogenous cytokinin, the surfactant used, the unpredictable pleiotropic nature of cytokinins and the effect of the environment and environmental stress. Given the evidence suggesting the importance of the genes expressed in the developing inflorescence (Ashikari *et al*., 2005; Yamburenko *et al*., 2017), and reports of effects when PGRs were applied earlier in development (personal communication, February 24, 2017), earlier growth stages, before anthesis and including when the inflorescence was developing, should be targeted in future experiments. Aside from these reasons, however, it appears most likely that the optimised growth conditions of these trials prevented further enhancement via the application of PGRs.

Application with TDZ-K reduced the content of chlorophyll in the primary leaf (an upper leaf) of wheat, and this effect contrasted with the reported anti-senescence properties of TDZ-K in detached leaf assays (United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript). Conversely, the general increase in the content of chlorophyll in the primary leaf following CPPU indicated the range of effects of each PGR, and the complexity of the mechanism and effect of each PGR, and highlighted the need for more work to elucidate these mechanisms. The limited effect of each PGR was most likely a result of the optimised field conditions. This led to a case for investigating the efficacy and effect of each PGR under various stress conditions, and these experiments are reported and discussed in Chapter 4.

Chapter 4
Wheat and barley pot trials

4.1 Introduction

4.1.1 Stress and senescence

Drought has a significant detrimental effect on global food production (Boyer, 1982), particularly when combined with other stress. There are major imminent challenges to the maintenance of food security including climate change (Nellemann *et al*., 2009), which can affect the presence of disease (IPCC, 2007) and the frequency of severe weather events including drought and flooding (IPCC, 2001; IPCC, 2007). This provides incentive for agricultural and technological advancements which can mitigate the detrimental effects of biotic and abiotic stress on crop plants.

Senescence is not an uncontrolled degenerative process but a controlled process involving programmed cell death, which allows for nutrient remobilisation from older leaves into younger, functionally productive photosynthesising leaves, fruits and seeds (Jordi *et al*., 2000). Senescence can be induced by environmental stresses including nutrient-deficient and drought stress conditions (Pourtau *et al*., 2004), and this stress response is part of the adaption of the plant to the environment. However, premature senescence is associated with a loss of biomass and/or yield in crop plants (Gepstein and Glick, 2013). Delaying senescence, particularly under stress conditions, is therefore an important target for yield enhancement as it would increase the photosynthetic capacity of leaves and increase the supply of photoassimilates to developing seeds (Masclaux-Daubresse *et al*., 2008; Rivero *et al*., 2009; Guiboileau *et al*., 2010).

4.1.2 Senescence and cytokinin

The role of cytokinin in stress response has been studied extensively (Yang *et al*., 2001; Brugière *et al*., 2003; Vyroubalová *et al*., 2009; Hare *et al*., 1997). Cytokinins are able to inhibit senescence-associated processes including chlorophyll and chloroplast protein degradation (Jordi *et al*., 2000; McCabe *et al*., 2001). The ability of cytokinins to delay senescence has been demonstrated with application experiments (Clarke *et al*., 1994; Noodén *et al*., 1997).

There are recent reports that INCYDE, which inhibits CKX activity (Zatloukal *et al*., 2008) and therefore, increases endogenous cytokinin, is able to alleviate symptoms and the effects of both abiotic and biotic stresses. This includes reducing the chlorotic and necrotic symptoms in arabidopsis infected with *Verticillium longisporum*, by spraying plants every three days (from four to 24 days after infection) with 10 µM INCYDE (Reusche *et al*., 2013), providing alleviation from effects of salt stress in tomatoes by foliar spraying 10 nM INCYDE (Aremu *et al*., 2014) and protection from cadmium stress following providing seeds of medicinal plants with 10 µM INCYDE (Gemrotová *et al*., 2013). There is little current research, however, on the effect of INCYDE on cereal crops during drought and nitrogen-limited conditions.

TDZ-K is known to not inhibit CKX, not inhibit wheat and arabidopsis root growth (when applied at 100 nM) and is able to inhibit wheat and barley leaf senescence in detached leaf assays (at concentration ranges between 0.1 to 100 µM) by blocking the degradation of photosynthetic complexes within photosystem II (J. Nisler, personal communication, August 28, 2017; United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript). Given the strong link between the delay of senescence (particularly under stress conditions) and yield enhancement (Gregersen *et al*., 2013; Guo and Gan, 2014), the efficacy of TDZ-K on wheat and barley plants subjected to different abiotic stresses was determined.

CPPU is well-known to inhibit CKX activity (Bilyeu *et al*., 2001; Nisler *et al*., 2016), and there is evidence to suggest that CPPU can have protective effects and delay senescence during drought with papaya when applied at around 120 µM (Jianchang *et al*., 2003) or under salt-stress conditions when 100 µM CPPU was applied to rice (Gashaw *et al*., 2014). Since its application can increase the concentration of endogenous cytokinin following inhibition of CKX (Chatfield and Armstrong, 1986; Laloue and Fox, 1989), it was hypothesised that CPPU might delay senescence and/or alleviate symptoms of abiotic stress.

A number of other approaches have been implemented to delay senescence including using various mutants or lines with senescence delaying traits, and these plants are categorised as 'stay-green' plants (Thomas and Howarth, 2000). A delay in senescence with 'stay-green' plants has been linked to enhanced yield in cereals including wheat, barley and rice (Gregersen *et al*., 2013, and references therein). Transgenic approaches using ectopic *IPT*-overexpression have been implemented successfully (Gan and Amasino, 1996). This includes the use of autoregulatory 'stay-green' systems using senescence-inducible promoters P_{SAG12} (Gan and Amasino, 1995) and P_{SARK} (Hajouj *et al*., 2000) to drive *IPT* expression. These are induced by local leaf senescence in plants, which occurs in older leaves first. This increase in cytokinin inhibits leaf senescence, and acts as a feedback system to inhibit the promoter and reduce further biosynthesis of cytokinin. This ensures that there is no excessive accumulation of cytokinins, which would otherwise result in abnormal growth (Guo and Gan, 2014). These experiments indicate that using transgenic crops where cytokinin was moderately increased in a highly regulated manner, was an effective method for ameliorating the effects of drought stress on yield in both monocot and dicot crop plants (Guo and Gan, 2014, and references therein).

4.1.3 Experimental aims

Field trials were previously carried out under optimised conditions (Chapter 3), and indicated that PGRs showed little evidence of delaying senescence or enhancing yield. Given previously described reports of cytokinin mitigating the impacts of stress on plants, it was hypothesised that an enhancement in cytokinin from applying CKX-inhibiting PGRs INCYDE and CPPU, and the application of TDZ-K which is able to inhibit senescence, through an unknown mechanism, would be able to ameliorate stress and/or provide an enhancement in yield under water-limited and nitrogen-limited conditions.

4.2 Materials and Methods

4.2.1 Outdoor pot trials

Pot trials were carried out across three seasons in 2013/14, 2014/15 and 2015/16 at the University of Canterbury, New Zealand (43°31'22.8"S 172°35'15.1"E) using spring wheat cultivar Morph (PGG Wrightson Grain) and barley cultivars Tavern and Fairview (PGG Wrightson Grain). These cultivars were suitable for growing in the pot trials during the summer. Pots were filled with fertiliser-free potting soil mix containing peat moss, perlite and pine bark. Each pot was 206 mm in height, 255 mm

in diameter at the widest point of the pot, and 109 mm diameter at the narrowest point on the pot. Each pot contained 4300 cm^3 (4.3 L) of soil. Several experiments were carried out, and each trial was given a designated name. Outdoor pot trials included a well-watered and nitrogen supplemented (see section **4.2.1.2** for the definition of nitrogen supplemented) pot trial for wheat (Trial 1) and nitrogen-limited trials which included wheat (Trial 2A) and barley (Trial 2B) (**Figure 4.1A**). Irrigation systems were placed around the outdoor pot trials and plants were irrigated once every one to three days based on the weather and demand, ensuring that plants were not subject to any water-deficiency. Netting was set up around the outdoor pots to prevent bird strike. A summary description of the pot trials is provided in **Table 4.1** and details of these trials is described in Appendix 4.1.

In glasshouses, nitrogen/water-limited trials were carried out and included wheat (Trial 3A) and barley (Trial 3B) (**Figure 4.1B**). Additional well-watered and nitrogen supplemented pot trials were carried out solely for chlorophyll analyses and involved wheat (Trial 4A) wheat and barley (Trial 4B).

4.2.1.1 Sowing rates

Each pot contained plants sown at a sowing rate between 60 to 103 kg/ha (Appendix 4.1). Following germination and appearance of the plants, each pot was standardised to eight plants per pot. The plant density and seed sowing depths were based on cereal recommendations (Photiades and Hadjichristodoulou, 1984; FAR, 2009; Hall, 2012; PGG Wrightson, 2015).

Table 4.1 Summary of each trial, the experimental conditions used and the purpose of each.

Trial name	Cultivar and year	Nitrogen-limited	Water-limited	Outdoor or glasshouse	Experimental purpose of pot trial
Wheat Trial 1	Morph 2013/14	NO	NO	Outdoor	This trial measured the yield and growth response of wheat to INCYDE and TDZ-K under well-watered conditions and provided with sufficient (but not optimised) nitrogen levels. Treatments were equivalent to that used in Orator wheat 2013/14 field trial (Chapter 3)
Wheat Trial 2A	Morph 2014/15	YES	NO	Outdoor	This trial measured the yield and growth response of wheat to CPPU and TDZ-K under well-watered and nitrogen-limited conditions. Treatments were equivalent to that used in Torch wheat 2014/15 field trial (Chapter 3)
Barley Trial 2B	Tavern 2014/15	YES	NO	Outdoor	This trial measured the yield and growth response of barley to INCYDE under well-watered and nitrogen-limited conditions. Treatments equivalent to that used in Quench barley 2014/15 field trial (Chapter 3)
Wheat Trial 3A	Morph 2014/15	YES	YES	Glasshouse	This trial measured the yield and growth response of wheat to INCYDE, TDZ-K and CPPU under water-limited and nitrogen-limited conditions in the glasshouse.
Barley Trial 3B	Tavern 2014/15	YES	YES	Glasshouse	This trial measured the yield and growth response of barley to INCYDE, TDZ-K and CPPU under water-limited and nitrogen-limited conditions in the glasshouse.
Wheat Trial 4A	Morph 2015/16	NO	NO	Outdoor	This trial was used to acquire samples to determine chlorophyll content in wheat leaves following the application of INCYDE, TDZ-K and CPPU.
Barley Trial 4B	Fairview 2015/16	NO	NO	Outdoor	This trial was used to acquire samples to determine chlorophyll content in barley leaves following the application of INCYDE and TDZ-K.

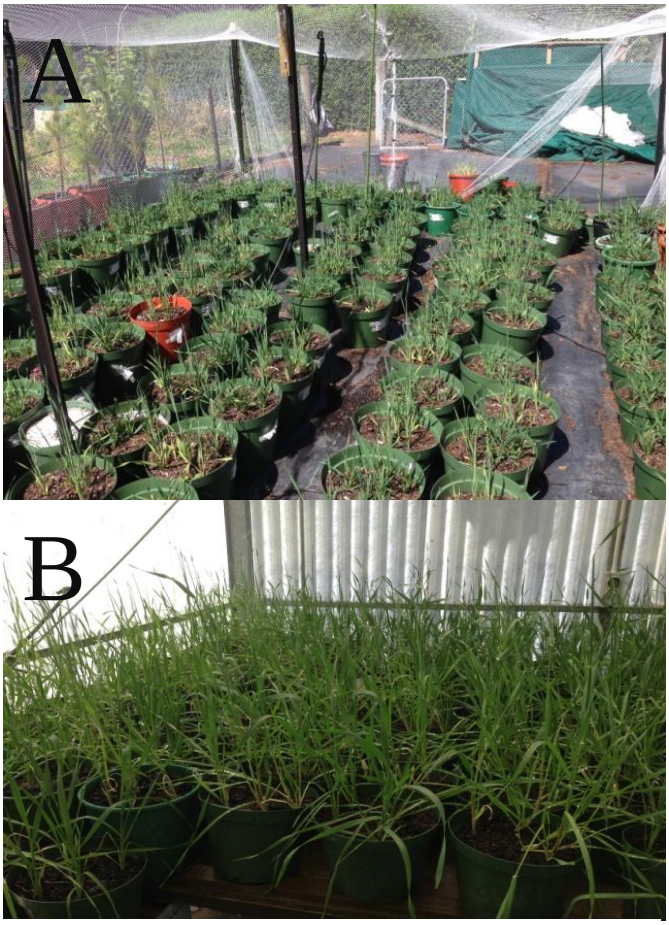

Figure 4.1 The experimental layout of pot trials. A: Outdoor pot trials with an irrigation system and netting; B: glasshouse trials. Trials were carried out at the University of Canterbury, New Zealand.

4.2.1.2 Nitrogen input

Nitrogen was supplied using slow release fertiliser Tui Novatec Premium Fertiliser (Tui Garden) (N (15%), P (1.3%), K (16.6%) + Mg and trace elements). Wheat Trial 1, wheat Trial 4A and barley Trial 4B were all provided with nitrogen rates of ≥ 220 kgN/ha split over sowing, early leaf development, tillering, stem elongation and anthesis (Appendix 4.1) based on nitrogen application guides by FAR (FAR, 2001; 2007; 2013). It is important to note that these trials were not nitrogen and nutrient optimised as the field trials were, in part because a proportion of the nitrogen added was lost through the base of the pots, and field trials had multiple applications of fertiliser over each season. These are referred to as 'nitrogen supplemented' pot trials. Conversely, nitrogen-limited pot trials wheat Trial 2A and barley Trial 2B, where nitrogen levels were even lower, were provided between 55 to 70 kgN/ha split over sowing, tillering and stem elongation.

4.2.1.3 Insect and fungi management

Fungi and insects were managed when required. Fungi were controlled with Yates Fungus Fighter (Yates) applied at 10 mL/L in water (v/v), while insects, including aphids, were controlled with Yates Pyrethrum Insecticide (Yates) applied at 5 mL/L in water (v/v).

4.2.1.4 Treatments

Treatment solutions were made by dissolving INCYDE, TDZ-K and CPPU in DMSO, and immediately prior to use, diluting the mix with water to produce working solutions between 10 and 100 µM. Positive control solutions were made up with DMSO/water mix equivalent to that used in 25, 50 or 100 µM treatments, while plants designated as 'Nil' were left untreated. Working solutions were mixed with 0.5% Yates Sprayfix (Yates) and applied by spraying whole plants until foliar runoff, using 500 mL spray bottles (McGregor's) at the same time each day. Each plant received 3 mL of treatment for each application at a given growth stage. A summary of the treatments used for yield and growth analyses in wheat Trial 1, wheat Trial 2A and barley Trial 2B is and the drought trials (Trial 3A and 3B) is provided in **Table 4.2**.

Table 4.2 A summary of outdoor pot trials. The treatments, concentrations and growth stages (Zadoks) targeted is described for wheat Trial 1, wheat Trial 2A (NL) and barley Trial 2B (NL) applied with 0.5% Yates Sprayfix.

Tmt. no.	Wheat Trial 1	Wheat Trial 2A (NL)	Barley Trial 2B (NL)
1	Nil	Nil	Nil
2	DMSO control (GS 61, 65, 65+2w)	DMSO control (GS 61, 65, 65+2w)	DMSO control (GS 39, 51, 61, 65)
3	DMSO control (GS 39, 51, 61, 65)	TDZ-K 10 µM (GS 61, 65, 65+2w)	INCYDE 25 µM (GS 39)
4	TDZ-K 10 µM (GS 61, 65, 65+2w)	TDZ-K 50 µM (GS 61, 65, 65+2w)	INCYDE 25 µM (GS 39, 51, 61, 65)
5	TDZ-K 25 µM (GS 61, 65, 65+2w)	CPPU 10 µM (GS 51, 65)	INCYDE 25 µM (GS 51)
6	INCYDE 10 µM (GS 65)	CPPU 10 µM (GS 61, 65)	INCYDE 10 µM (GS 65)
7	INCYDE 25 µM (GS 39, 51, 61, 65)	CPPU 30 µM (GS 51, 65)	INCYDE 25 µM (GS 61)
8	INCYDE 25 µM (GS 39)	CPPU 30 µM (GS 61, 65)	INCYDE 25 µM (GS 65)
9	INCYDE 25 µM (GS 51)	CPPU 100 µM (GS 51, 65)	INCYDE 50 µM (GS 61)
10	INCYDE 25 µM (GS 61)	CPPU 100 µM (GS 61, 65)	
11	INCYDE 25 µM (GS 65)		
12	INCYDE 50 µM (GS 61)		
13	INCYDE 50 µM (GS 65)		

4.2.2 Glasshouse pot trials

Glasshouse pot trials were carried out using the pots and fertiliser-free potting mix used for the outdoor pot trials (**Figure 4.1B**). Glasshouse temperatures were maintained within a minimum of 12ºC and a maximum of 30°C, while the humidity and light were subject to prevailing conditions. Artificial lighting systems were used when the natural light period was less than 16 h, and maintained between 7 am and 11 pm at ~ 500 µmol $m^{-2}s^{-1}$, later in development when the photoperiod declined.

Wheat was sown at a rate of 48.3 kg/ha and barley was sown at 50.4 kg/ha at a depth of 3 cm (Appendix 4.1). Pots were thinned down to eight plants per pot following germination and appearance of the plants. Wheat was provided with 69 kgN/ha and barley 57 kgN/ha split over sowing and tillering using Tui Novatec Premium Fertiliser (Tui Garden). Plants were irrigated once every two days until GS 51, when a period of drought was initiated for 20 d until plants showed symptoms of water stress including widespread senescence and delayed anthesis. This was followed by the resumption of normal irrigation rates. Working solutions of 50 µM INCYDE, 50 µM TDZ-K and 100 µM CPPU were applied once until runoff at 18 d from the initiation of drought period. These concentrations are within the range used in previous experiments for each PGR, as described in section **4.1.2**.

4.2.3 Experimental design and analyses

For both outdoor and glasshouse pot trials, pots were arranged in a randomised complete block design (**Figure 4.2**), with pots arranged into three different blocks, except for Trial 4A and 4B, which were arranged into four blocks for chlorophyll content analyses. For each treatment level, there were six pots within each block, and each pot had seeds sown at rates previously described (section **4.2.1.1**). There were six pots for each treatment level replicate, and each treatment level was represented once in each block. Replicates for a treatment level were separated by a buffer zone to remove any chance of treatment crossover.

4.2.3.1 Growth and yield measurements and analyses

Following the total senescence of wheat and barley in each trial, measurements were made of components of growth and yield. Up to ten randomly acquired whole plant samples were removed from each replicate for each treatment level, and the tillers were divided into large/main stems and small/tiller stems (**Figure 4.2**). Components of yield were measured for both the large and small

tillers. The stem length was measured as the length from the point of connection to the head, while the stem diameter was measured at the first node above the soil line. The head length was measured from the point of connection to the stem to the highest point of the upper most spikelet. Samples that were acquired for dry weight (DW) and yield measurements were placed in ovens for 2 h at 70 to 80°C until weight stabilisation immediately prior to DW and yield measurements. The head DW was measured for large and small tillers.

For each replicate of a treatment level, for both the large and small tillers, ten randomly acquired grains were taken from the heads for wheat and barley (**Table 4.3**). With the barley drought trial (Trial 4B), for each replicate, 30 grains were taken from barley heads, as there was high variability between the weight of individual grains. The thousand grain weight (TGW) measurements were taken overall for wheat and barley and at positions 1 to 2 for the heads of wheat (positions are displayed in **Figure 3.3C**). For the barley drought trial (Trial 4B) for each replicate, the number of grains per head was counted for 10 heads. A summary of the number of whole wheat and barley plants, as well as subsamples for each trait used following each pot trial harvest is provided in **Table 4.3** and in the caption of each result table.

The number of samples taken from each replicate was equal for each treatment level, and for each treatment level the mean was calculated for each treatment level replicate. The treatment level overall mean was calculated using the three replicates (**Figure 4.2**), and along with the standard error, reported in the results tables. Less whole plants were able to be acquired from Trial 3B and Trial 2A (**Table 4.3**) compared to other trials, due to bird strike, harsh conditions or aphid or fungi infection, all of which reduced the number of plants available for harvest.

Table 4.3 A summary of the subsample numbers taken from each replicate for each treatment level for yield and growth traits of wheat and barley pot trials.

		For each replicate of each treatment level			
Trial	No. blocks	No. of whole plants acquired	Number of heads acquired for measuring head length, DW and no. grains per head	Number of stems acquired for measuring stem length, diameter and DW	Number of grains acquired for measuring TGW overall and at positions 1 to 2
Wheat Trial 1	3	10	10	10	10
Wheat Trial 2A	3	5	5	N/A	10
Barley Trial 2B	3	10	10	N/A	10
Drought wheat Trial 3A	3	10	10	10	30
Drought barley Trial 3B	3	8	8	8	10

N/A not applicable: no measurements were made for these traits for these trials. Less whole plants were acquired from Trial 3B and Trial 2A due to bird strike, harsh conditions or aphid or fungi infection.

Using the treatment replicate means calculated for each trait within each pot trial, to determine whether there was a statistically significant difference between means, an ANOVA (significance level: 0.05, two sided) was carried out using Minitab 17 (Minitab Inc.), partitioning for the treatment factor and the block factor. All factors were fixed. For the outdoor pot trials, when the *p*-value was $\leq$ 0.05 for the treatment factor, a *post hoc* Tukey HSD test was carried out (confidence interval: 95%) using XLSTAT 2016 (Addinsoft), and reported in the Appendix referenced in each result table. In the case of the drought trials, there was only one control group, with only pairwise comparisons necessary with the single control, therefore, when the *p*-value was ≤ 0.05 for the treatment factor, a *post hoc* two sided Dunnett test (confidence interval: 95%) was carried out using XLSTAT 2016 (Addinsoft) to determine if there was a statistically significant difference between the control and treatments. To determine if the assumptions of the ANOVA were met, residual plots of the standardised residual were generated with Minitab 17 and reported in the Appendix. A Q-Q plot was carried out to determine if residuals were normally distributed and a plot of the standardised residuals against predicted values was examined to determine equality of the variances. A Levene's test was also additionally carried out to provide certainty of the equality of the variances and was provided in the notes of the results table (in addition to a residual plot in the Appendix), for results where there was a statistically significant difference between a treatment and the controls. For the number of grains per head, since this data was count data, a Poisson regression (Log-link function, confidence interval: 95%, Two-sided) was carried out using Minitab 17, with the DMSO control set as the reference level. The coefficients and *p*-values were examined.

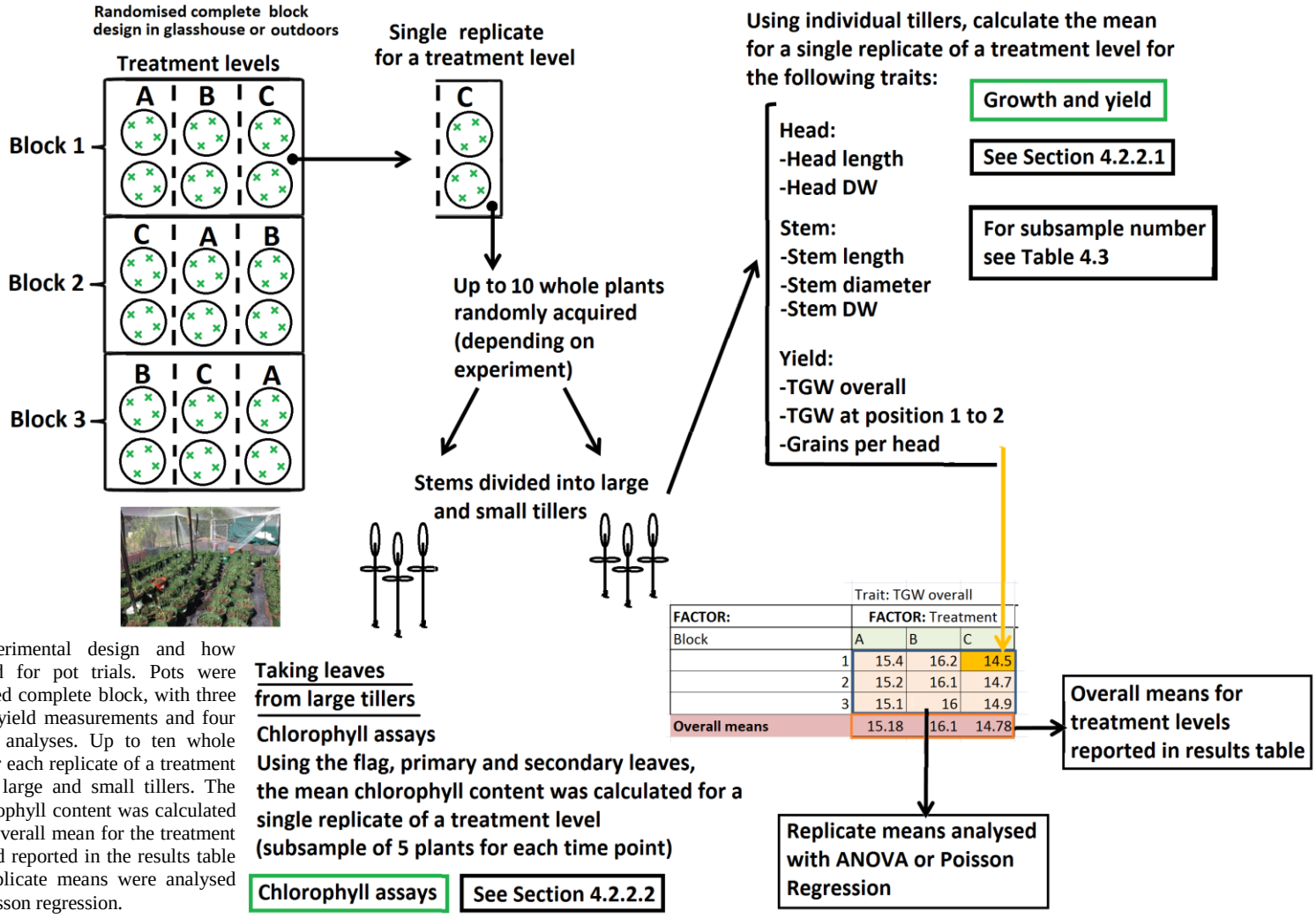

Figure 4.2 The experimental design and how samples were acquired for pot trials. Pots were arranged in a randomised complete block, with three blocks for growth and yield measurements and four blocks for chlorophyll analyses. Up to ten whole plants were acquired for each replicate of a treatment level and divided into large and small tillers. The growth, yield and chlorophyll content was calculated from these tillers. The overall mean for the treatment level was calculated and reported in the results table while the treatment replicate means were analysed with an ANOVA or Poisson regression.

4.2.3.2 Chlorophyll content analyses

For chlorophyll assays, wheat (Trial 4A) and barley (Trial 4B) (**Table 4.1**) were grown and arranged in a randomised complete block trial, with four blocks and each treatment level represented once in each block. At each time point, for each replicate of a treatment level, five whole plant samples were acquired. For the wheat trial, flag, primary and secondary leaves were acquired following treatments at GS 39, 51 and 61with INCYDE, TDZ-K and CPPU (defined as week 0), and then subsequently at two week intervals up to eight weeks when leaves had undergone complete senescence and/or fallen off. In barley Trial 4B flag, primary and secondary leaves were acquired following INCYDE or TDZ-K treatment at GS 51 and 61 and subsequently at two week intervals up to six weeks. Leaf samples were immediately placed onto ice and transported to the lab to be stored at - 20°C until analysis. Samples measuring 1 cm x 1 cm were cut out of the middle of the barley and wheat leaves. Replicates were between 0.7 and 54 mg fresh weight and were added to 300 µL dimethylformamide (DMF) (Sigma-Aldrich) to produce solutions up to 0.18 mg FW/µL DMF and analysed as described in section **2.2.5**. The mean chlorophyll content was calculated for each replicate, and the overall mean for the four replicates calculated and reported along with the standard error in the results tables.

To determine if there were significant differences in the means of the chlorophyll content, an ANOVA (significance level: 0.05, two sided) was carried out (Minitab 17) partitioning for the treatment factor, the time factor (when samples were acquired), the interaction between treatment and time (time*treatments) and the block factor. All factors were fixed. Prior to ANOVA, using Minitab 17, residual plots were produced to establish that residuals were normally distributed, and to ensure that the variances were homoscedastic, and each of these plots were reported in the Appendix. Following an ANOVA, where the *p*-value was ≤ 0.05 for a factor or interaction, a *post hoc* Tukey HSD test (confidence interval: 95%) was used to establish if there were statistically significant differences between treatments and the control (XLSTAT 2016). Where there was a *p*-value ≤ 0.05 for the interaction, an interaction plot was carried out (Minitab 17) and reported in the Appendix. The effect of treatments overall and at specific time points were both determined. Where statistically significant differences existed between the control and a treatment at a specific time point, this was indicated with an * next to the chlorophyll content value that was provided (in mg/g). For significant differences between a treatment and control (e.g. a pairwise comparison of INCYDE vs. control), this was indicated with a ** next to the treatment.

4.3 Results

4.3.1 Wheat pot trial (Trial 1)

Wheat pot trial (Trial 1) was carried out to determine the effect of PGRs on wheat in pot trials, this experiment replicated the treatments used in the wheat Orator (2013/14) field trial in Chapter 3. With wheat pot trial (Trial 1), analyses of variance showed that there were no statistically significant difference between the treatment means for the head length, head DW and stem length for large and small tillers (**Table 4.4**). For thousand grain weight (TGW) measurements with Trial 1 (**Table 4.5**), the *p*-value for the treatment factor was < 0.05 for the TGW overall for large ($F_{12, 24}$ = 3.04, p = 0.01) and small tillers ($F_{12, 24}$ = 5.14, p < 0.001), and at positions 1 to 2 in large ($F_{12, 24}$ = 3.25, p = 0.007) and small tillers ($F_{12, 24}$ = 2.9, p = 0.013). However, in each case, a *post hoc* Tukey HSD test (confidence interval: 95%) revealed that there was no statistically significant difference between a treatment level and all of the controls (see Appendices 4.2.7 to 4.2.10).

4.3.2 Nitrogen-limited trials (Trial 2A and 2B)

Nitrogen-limited wheat and barley trials (Trial 2A and 2B) were carried out to determine the effect of PGRs under stressed (nitrogen-limited) conditions. The treatments here replicated the treatments used in 2014/15 Torch wheat and Quench barley field trials. With the nitrogen-limited wheat pot trial (Trial 2A), following 30 µM CPPU (GS 61, 65) treatment, there was a statistically significant decrease in the TGW overall of small tillers ($F_{9, 18}$ = 7.35, p < 0.001, *post hoc* Tukey HSD, confidence interval: 95%, Appendix 4.3.6) and at position 1 and 2 in small tillers ($F_{9, 18}$ = 7.56, p < 0.001, *post hoc* Tukey HSD, confidence interval: 95%, Appendix 4.3.8) (**Table 4.6**). There was no statistically significant difference between the treatments and the controls in the TGW overall of large tillers and at position 1 to 2 in large tillers, nor in the head length and head DW of both large and small tillers.

In the nitrogen-limited barley pot trial (Trial 2B), there was a statistically significant decrease in the large head DW of barley plants treated with 25 µM INCYDE (GS 65) ($F_{8, 16}$ = 5.9, p = 0.001, *post hoc* Tukey HSD, confidence interval: 95%, Appendix 4.4.3) (**Table 4.7**). There was no corresponding change in the head length or TGW overall for this treatment, nor any other statistically significant difference in the head length, head DW or TGW overall.

Table 4.4 The wheat pot trial (Trial 1) head length, head dry weight and stem length for large and small tillers following INCYDE and TDZ-K treatment.

Treatment	Large Head Length (mm)	Small Head Length (mm)	Large Head DW (g)	Small Head DW (g)	Large Stem Length (mm)	Small Stem Length (mm)
Nil	77.4 ± 1.1	71.5 ± 1.5	1.6 ± 0.08	1.2 ± 0.1	509.5 ± 15.6	476.2 ± 1.1
DMSO Control (GS 39, 51, 60, 65)	80.3 ± 1.9	69.1 ± 0.7	1.7 ± 0.07	1.1 ± 0.09	512.3 ± 7.0	483.2 ± 7.8
DMSO Control (GS 61, 65+2W)	78.7 ± 2.8	67.5 ± 1.1	1.6 ± 0.09	1.2 ± 0.05	521.0 ± 3.8	461.4 ± 18.3
INCYDE 10 µM (GS 65)	80.2 ± 1.3	70.5 ± 1.3	1.7 ± 0.03	1.1 ± 0.03	522.3 ± 18.2	502.1 ± 7.7
INCYDE 25 µM (GS 39, 51, 60, 65)	79.6 ± 2.5	71.4 ± 0.3	1.7 ± 0.06	1.1 ± 0.07	524.4 ± 6.6	487.9 ± 10.3
INCYDE 25 µM (GS 39)	79.5 ± 2.0	68.4 ± 2.2	1.8 ± 0.06	1.0 ± 0.08	531.7 ± 14.7	489.1 ± 16.6
INCYDE 25 µM (GS 51)	79.6 ± 0.9	70.6 ± 1.6	1.8 ± 0.02	1.1 ± 0.05	522.7 ± 8.4	500.4 ± 21.0
INCYDE 25 µM (GS 61)	79.3 ± 1.0	71.9 ± 0.1	1.8 ± 0.2	1.3 ± 0.03	539.0 ± 10.0	521.6 ± 14.6
INCYDE 25 µM (GS 65)	81.0 ± 1.9	73.9 ± 1.0	1.6 ± 0.04	1.2 ± 0.04	514.6 ± 15.2	515.5 ± 9.3
INCYDE 50 µM (GS 61)	79.1 ± 1.8	71.2 ± 1.4	1.8 ± 0.1	1.2 ± 0.07	525.1 ± 9.8	503.9 ± 14.9
INCYDE 50 µM (GS 65)	78.2 ± 0.6	72.8 ± 1.5	1.6 ± 0.03	1.1 ± 0.05	525.7 ± 7.3	463.9 ± 9.9
TDZ-K 10 µM (GS 61, 65+2W)	78.8 ± 0.9	71.8 ± 1.8	1.7 ± 0.08	1.1 ± 0.07	524.1 ± 4.9	493.4 ± 8.7
TDZ-K 25 µM (GS 61+65+2W)	81.1 ± 1.5	66.9 ± 1.5	1.7 ± 0.07	1.1 ± 0.05	523.7 ± 2.5	489.9 ± 11.5
Treatment *F*-value ($F_{12, 24}$)[a]	0.4	2.17	1.39	1.72	0.69	1.89
Treatment *p*-value	0.948	0.052	0.235	0.125	0.741	0.089
Statistics	**Appendix 4.2.1**	**Appendix 4.2.2**	**Appendix 4.2.3**	**Appendix 4.2.4**	**Appendix 4.2.5**	**Appendix 4.2.6**
Analysis notes	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).
Trial 1 was an outdoor pot trial carried out with wheat cultivar Morph over the 2013/14 season. The pot trial was neither nitrogen or water-limited. The overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates (n = 3), within separate blocks, arranged in a randomised complete block trial (see section **4.2.3** and **Figure 4.2**). For each treatment level replicate, ten whole plants were acquired (30 in total for a treatment level) and plants divided into large and small tillers as described in section **4.2.3.1**. See **Table 4.3** for a summary of the subsample numbers for each trait measured. The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **4.2.1.4**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al.*, 1974). Tests including ANOVA and residual plots for the ANOVA assumptions are provided in **Appendices 4.2.1** to **4.2.6** for each trait, and *F*-values and *p*-values are provided. For more information on the experimental design and analyses see section **4.2.3.1**.

Table 4.5 The wheat pot trial (Trial 1) thousand grain weight (TGW) and TGW at position 1 to 2 for large and small tillers following INCYDE and TDZ-K treatment.

Treatment	Large TGW Overall (g)	Small TGW Overall (g)	Large TGW Pos 1-2 (g)	Small TGW Pos 1-2 (g)
Nil	33.0 ± 0.6	35.4 ± 1.0	35.1 ± 0.9	35.9 ± 1.5
DMSO Control (GS 39, 51, 60, 65)	33.1 ± 0.2	33.4 ± 0.7	35.7 ± 0.3	35.1 ± 0.8
DMSO Control (GS 61, 65+2W)	35.2 ± 0.9	32.5 ± 1.3	38.2 ± 0.7	37.9 ± 1.0
INCYDE 10 µM (GS 65)	30.8 ± 1.1	32.0 ± 0.5	33.3 ± 0.9	34.3 ± 0.1
INCYDE 25 µM (GS 39, 51, 60, 65)	31.0 ± 1.0	31.3 ± 1.1	33.2 ± 1.3	34.9 ± 1.1
INCYDE 25 µM (GS 39)	30.1 ± 0.3	32.2 ± 1.3	33.9 ± 0.3	35.1 ± 0.7
INCYDE 25 µM (GS 51)	33.0 ± 1.1	35.2 ± 0.7	36.4 ± 1.9	36.5 ± 0.5
INCYDE 25 µM (GS 61)	35.3 ± 1.7	36.6 ± 1.4	37.7 ± 0.6	38.6 ± 1.3
INCYDE 25 µM (GS 65)	30.0 ± 1.8	35.4 ± 0.3	32.4 ± 1.9	38.5 ± 0.7
INCYDE 50 µM (GS 61)	31.7 ± 0.2	37.4 ± 1.0	34.3 ± 0.1	37.4 ± 1.0
INCYDE 50 µM (GS 65)	33.8 ± 0.6	33.6 ± 0.4	35.8 ± 1.5	35.9 ± 0.6
TDZ-K 10 µM (GS 61, 65+2W)	34.6 ± 1.2	30.2 ± 1.0	38.1 ± 1.1	34.2 ± 0.8
TDZ-K 25 µM (GS 61+65+2W)	32.9 ± 0.9	33.4 ± 0.6	34.4 ± 1.0	37.0 ± 1.1
Treatment *F*-value ($F_{12, 24}$)[a]	3.04	5.14	3.25	2.9
Treatment *p*-value	**0.01**	**< 0.001**	**0.007**	**0.013**
Statistics	**Appendix 4.2.7**	**Appendix 4.2.8**	**Appendix 4.2.9**	**Appendix 4.2.10**
Analysis notes	ANOVA, Tukey HSD	ANOVA, Tukey HSD	ANOVA, Tukey HSD	ANOVA, Tukey HSD

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

Trial 1 was an outdoor pot trial carried out with wheat cultivar Morph over the 2013/14 season. The pot trial was neither nitrogen or water-limited. The overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates (n = 3), within separate blocks, arranged in a randomised complete block trial (see section **4.2.3** and **Figure 4.2**). For each treatment level replicate, ten whole plants were acquired (30 in total for a treatment level), divided into large and small tillers, and ten grains (30 in total for a treatment level) were randomly acquired at different positions as described in section **4.2.3.1**. See **Table 4.3** for a summary of the subsample numbers for each trait measured. The 'Nil' treatment represents no treatment, while DMSO controls were produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **4.2.1.4**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA, Tukey HSD and residual plots for the ANOVA assumptions are provided in **Appendices 4.2.7** to **4.2.10** for each trait, and *F*-values and *p*-values are provided. For more information on the experimental design and analyses see section **4.2.3.1**.

Table 4.6 The nitrogen-limited wheat pot trial (Trial 2A) head length, head dry weight (DW), thousand grain weight (TGW) overall and at positions 1 to 2 in large and small tillers following TDZ-K and CPPU treatment.

Treatment	Large Head Length (mm)	Small Head Length (mm)	Large Head DW (g)	Small Head DW (g)	Large TGW Overall (g)	Small TGW Overall (g)	Large TGW Pos 1-2 (g)	Small TGW Pos 1-2 (g)
DMSO Control (GS 61, 65,+2W)	76.4 ± 2.3	65.1 ± 2.0	1.9 ± 0.1	1.2 ± 0.1	38.5 ± 1.8	37.2 ± 0.6	40.8 ± 2.5	39.2 ± 0.3
Nil	77.0 ± 1.5	62.8 ± 4.2	1.7 ± 0.1	1.1 ± 0.1	37.3 ± 2.0	36.2 ± 0.7	39.4 ± 2.4	38.6 ± 0.7
TDZ-K 10 µM (GS 61,65+2W)	74.8 ± 2.2	62.2 ± 4.0	1.9 ± 0.1	1.3 ± 0.1	40.3 ± 1.6	35.9 ± 1.4	41.7 ± 2.1	39.2 ± 1.2
TDZ-K 50 µM (GS 61,65+2W)	74.1 ± 1.0	62.9 ± 0.4	1.8 ± 0.1	1.3 ± 0.1	38.4 ± 3.4	35.8 ± 0.5	41.4 ± 3.9	38.5 ± 0.5
CPPU 10 µM (GS 51, 65)	77.4 ± 4.1	57.2 ± 1.2	1.8 ± 0.1	1.1 ± 0.05	36.8 ± 1.2	37.5 ± 0.6	39.9 ± 2.3	39.7 ± 0.4
CPPU 30 µM (GS 51, 65)	73.9 ± 4.3	61.7 ± 1.8	1.7 ± 0.1	1.2 ± 0.05	36.9 ± 2.3	37.1 ± 0.7	38.9 ± 3.4	40.3 ± 0.4
CPPU 100 µM (GS 51, 65)	69.8 ± 4.1	63.2 ± 3.2	1.6 ± 0.1	1.1 ± 0.1	33.8 ± 2.0	36.2 ± 0.5	37.1 ± 2.1	39.0 ± 0.9
CPPU 10 µM (GS 61, 65)	78.2 ± 2.8	61.2 ± 0.7	1.8 ± 0.1	1.3 ± 0.1	40.2 ± 1.8	36.9 ± 0.5	41.1 ± 2.7	39.8 ± 0.1
CPPU 30 µM (GS 61, 65)	77.8 ± 0.7	62.8 ± 2.7	1.8 ± 0.1	1.1 ± 0.1	37.8 ± 1.4	**32.9 ± 0.6***	41.2 ± 2.0	**35.2 ± 0.1***
CPPU 100 µM (GS 61, 65)	75.2 ± 2.1	61.1 ± 2.1	1.9 ± 0.1	1.3 ± 0.1	36.4 ± 2.9	38.0 ± 0.5	39.1 ± 3.3	40.9 ± 0.3
Treatment *F*-value ($F_{9, 18}$)[a]	1.05	0.8	0.99	1.99	3.26	7.35	2.3	7.56
Treatment *p*-value	0.442	0.62	0.479	0.103	**0.016**	**< 0.001**	0.064	**< 0.001**
Statistics	**Appendix 4.3.1**	**Appendix 4.3.2**	**Appendix 4.3.3**	**Appendix 4.3.4**	**Appendix 4.3.5**	**Appendix 4.3.6**	**Appendix 4.3.7**	**Appendix 4.3.8**
Analysis notes	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA, Tukey HSD	ANOVA, Tukey HSD, Levene's *p*-value: 0.867	ANOVA	ANOVA, Tukey HSD, Levene's *p*-value: 0.599

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

* Indicates a statistically significant difference (Tukey HSD, confidence interval: 95%) for a treatment compared to both the DMSO control and the 'Nil' control.

Trial 2A was an outdoor pot trial carried out with wheat cultivar Morph over the 2014/15 season. The pot trial was nitrogen-limited. The overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates (n = 3), within separate blocks, arranged in a randomised complete block trial (see section **4.2.3** and **Figure 4.2**). For each treatment level replicate, five whole plants were acquired (15 in total for a treatment level), divided into large and small tillers, and ten grains (30 in total for a treatment level) were randomly acquired at different positions as described in section **4.2.3.1**. See **Table 4.3** for a summary of the subsample numbers for each trait measured. The 'Nil' treatment represents no treatment, while DMSO control was produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **4.2.1.4**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA, Tukey HSD and residual plots for the ANOVA assumptions are provided in **Appendices 4.3.1** to **4.3.8** for each trait, and *F*-values and *p*-values are provided. For more information on the experimental design and analyses see section **4.2.3.1**.

Table 4.7 The nitrogen-limited barley pot trial (Trial 2B) head length, head dry weight (DW) and thousand grain weight (TGW) overall in large and small tillers following INCYDE treatment.

Treatment	Large Head Length (mm)	Small Head Length (mm)	Large Head DW (g)	Small Head DW (g)	Large TGW Overall (g)	Small TGW Overall (g)
DMSO Control (GS 39, 51, 61, 65)	62.0 ± 1.4	52.0 ± 0.6	0.9 ± 0.06	0.6 ± 0.03	53.5 ± 0.5	49.9 ± 2.4
INCYDE 25 µM (GS 51)	59.9 ± 0.9	50.8 ± 0.9	0.9 ± 0.02	0.6 ± 0.004	54.3 ± 0.3	48.9 ± 0.9
INCYDE 25 µM (GS 65)	54.5 ± 2.1	43.8 ± 1.2	**0.6 ± 0.07***	0.5 ± 0.05	50.5 ± 2.1	46.0 ± 1.0
INCYDE 25 µM (GS 39, 51, 61, 65)	67.5 ± 3.3	53.2 ± 2.9	0.9 ± 0.03	0.5 ± 0.03	52.2 ± 0.7	46.7 ± 0.6
INCYDE 25 µM (GS 61)	56.1 ± 2.0	43.7 ± 0.9	0.8 ± 0.05	0.5 ± 0.04	51.4 ± 0.5	48.4 ± 1.0
INCYDE 50 µM (GS 65)	61.3 ± 2.8	49.0 ± 1.2	0.8 ± 0.05	0.5 ± 0.04	54.3 ± 2.3	44.5 ± 2.6
INCYDE 10 µM (GS 65)	60.3 ± 0.6	50.1 ± 1.3	0.9 ± 0.06	0.6 ± 0.05	52.3 ± 0.02	51.7 ± 0.4
INCYDE 25 µM (GS 39)	69.2 ± 1.8	51.0 ± 1.4	1.1 ± 0.05	0.6 ± 0.06	54.8 ± 0.7	47.3 ± 0.1
Nil	61.6 ± 0.6	47.9 ± 2.2	0.9 ± 0.02	0.6 ± 0.02	54.8 ± 0.6	51.7 ± 0.9
Treatment *F*-value ($F_{8, 16}$)[a]	5.66	5.05	5.9	3.07	1.82	3.7
Treatment *p*-value	**0.002**	**0.003**	**0.001**	**0.027**	0.147	**0.013**
Statistics	**Appendix 4.4.1**	**Appendix 4.4.2**	**Appendix 4.4.3**	**Appendix 4.4.4**	**Appendix 4.4.5**	**Appendix 4.4.6**
Analysis notes	ANOVA, Tukey HSD	ANOVA, Tukey HSD	ANOVA, Tukey HSD, Levene's *p*-value: 0.994	ANOVA, Tukey HSD	ANOVA	ANOVA, Tukey HSD

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

* Indicates a statistically significant difference (Tukey HSD, confidence interval: 95%) for a treatment compared to both the DMSO control and the 'Nil' control.

Trial 2B was an outdoor pot trial carried out with barley cultivar Tavern over the 2014/15 season. The pot trial was nitrogen-limited. The overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates (n = 3), within separate blocks, arranged in a randomised complete block trial (see section **4.2.3** and **Figure 4.2**). For each treatment level replicate, ten whole plants were acquired (30 in total for a treatment level), divided into large and small tillers, and ten grains (30 in total for a treatment level) were randomly acquired at different positions as described in section **4.2.3.1**. See **Table 4.3** for a summary of the subsample numbers for each trait measured. The 'Nil' treatment represents no treatment, while DMSO control was produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **4.2.1.4**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA, Tukey HSD and residual plots for the ANOVA assumptions are provided in **Appendices 4.4.1** to **4.4.6** for each trait, and *F*-values and *p*-values are provided. For more information on the experimental design and analyses see section **4.2.3.1**.

4.3.3 Drought trials (Trial 3A and 3B)

The glasshouse drought trials (Trial 3A and 3B) were carried out to determine the effect of each PGR (INCYDE, TDZ-K and CPPU) on wheat and barley under water-limited conditions. For the drought wheat trial (Trial 3A), there was a statistically significant decrease in the DW of heads of small tillers following 50 µM INCYDE treatment compared to the control ($F_{3, 6}$ = 7.03, p = 0.022, *post hoc* Dunnett test, two sided, confidence interval: 95%, Appendix 4.5.4) (**Table 4.8**), but no significant difference between a treatment and the control in large head DW, or the head length in small and large tillers.

Compared to the control, there was also a statistically significant decrease in the diameter of stems following 50 µM TDZ-K treatment in large tillers ($F_{3, 6}$ = 8.83, p = 0.013, *post hoc* Dunnett test, two sided, confidence interval: 95%, Appendix 4.5.5) (**Table 4.9**). There was no significant difference between the treatments and controls of the stem diameter of small tillers or the TGW overall for both large and small tillers.

In the drought barley trial (Trial 3B), there was a statistically significant decrease in the TGW overall following 100 µM CPPU treatment compared to the control in both large ($F_{3, 6}$ = 7.59, p = 0.018, *post hoc* Dunnett test, two sided, confidence interval: 95%, Appendix 4.6.5) (**Table 4.11**) and small tillers ($F_{3, 6}$ = 16.2, p = 0.003, *post hoc* Dunnett test, two sided, confidence interval: 95%, Appendix 4.6.6). There was no statistically significant difference between any of the treatments and control group in both large and small tillers in the stem length, stem DW (**Table 4.10**), and the number of grains per head (**Table 4.11**).

Table 4.8 The drought wheat pot trial (Trial 3A) head length and head dry weight (DW) in large and small tillers following INCYDE, TDZ-K and CPPU treatment.

Treatment	Large Head Length (mm)	Small Head Length (mm)	Large Head DW (g)	Small Head DW (g)
Control	96.5 ± 2.5	87.0 ± 2.6	1.2 ± 0.08	1.0 ± 0.07
INCYDE 50 µM	97.5 ± 1.3	79.3 ± 2.5	1.1 ± 0.02	**0.6 ± 0.04***
TDZ-K 50 µM	94.3 ± 1.2	81.1 ± 4.2	1.1 ± 0.08	0.8 ± 0.06
CPPU 100 µM	96.6 ± 1.8	84.6 ± 4.9	1.4 ± 0.01	0.8 ± 0.003
Treatment *F*-value ($F_{3, 6}$)[a]	0.52	0.79	2.56	7.03
Treatment *p*-value	0.682	0.543	0.15	**0.022**
Statistics	**Appendix 4.5.1**	**Appendix 4.5.2**	**Appendix 4.5.3**	**Appendix 4.5.4**
Analysis notes	ANOVA	ANOVA	ANOVA	ANOVA, Dunnett test, Levene's *p*-value: 0.61

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).
* Indicates a statistically significant difference (Dunnett, two sided, confidence interval: 95%) for a treatment compared to the control.
Trial 3A was a glasshouse pot trial carried out with wheat cultivar Morph over the 2014/15 season. The pot trial was nitrogen-limited and water-limited. The overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates (n = 3), within separate blocks, arranged in a randomised complete block trial (see section **4.2.3** and **Figure 4.2**). For each treatment level replicate, ten whole plants were acquired (30 in total for a treatment level) and plants divided into large and small tillers as described in section **4.2.3.1**. See **Table 4.3** for a summary of the subsample numbers for each trait measured. The control was produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **4.2.1.4**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA, Dunnett test and residual plots for the ANOVA assumptions are provided in **Appendices 4.5.1** to **4.5.4** for each trait, and *F*-values and *p*-values are provided. For more information on the experimental design and analyses see section **4.2.3.1**.

Table 4.9 The drought wheat pot trial (Trial 3A) stem diameter and thousand grain weight (TGW) overall in large and small tillers following INCYDE, TDZ-K and CPPU treatment.

Treatment	Large Stem Diameter (mm)	Small Stem Diameter (mm)	Large TGW Overall	Small TGW Overall (g)
Control	2.6 ± 0.1	2.3 ± 0.1	22.7 ± 3.7	29.2 ± 3.7
INCYDE 50 µM	2.7 ± 0.1	2.7 ± 0.03	22.3 ± 3.3	23.7 ± 0.9
TDZ-K 50 µM	**2.2 ± 0.02***	2.7 ± 0.08	23.2 ± 3.8	23.1 ± 3.9
CPPU 100 µM	2.6 ± 0.05	2.6 ± 0.09	24.8 ± 3.3	30.8 ± 0.8
Treatment *F*-value ($F_{3, 6}$)[a]	8.83	2.9	4.28	3.4
Treatment *p*-value	**0.013**	0.124	0.062	0.094
Statistics	**Appendix 4.5.5**	**Appendix 4.5.6**	**Appendix 4.5.7**	**Appendix 4.5.8**
Analysis notes	ANOVA, Dunnett test, Levene's *p*-value: 0.419	ANOVA	ANOVA	ANOVA

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).
* Indicates a statistically significant difference (Dunnett, two sided, confidence interval: 95%) for a treatment compared to the control.
Trial 3A was a glasshouse pot trial carried out with wheat cultivar Morph over the 2014/15 season. The pot trial was nitrogen-limited and water-limited. The overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates (n = 3), within separate blocks, arranged in a randomised complete block trial (see section **4.2.3** and **Figure 4.2**). For each treatment level replicate, ten whole plants were acquired (30 in total for a treatment level) divided into large and small tillers, and 30 grains (90 in total for a treatment level) were randomly acquired at different positions as described in section **4.2.3.1**. See **Table 4.3** for a summary of the subsample numbers for each trait measured. The control was produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **4.2.1.4**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA, Dunnett test and residual plots for the ANOVA assumptions are provided in **Appendices 4.5.5** to **4.5.8** for each trait, and *F*-values and *p*-values are provided. For more information on the experimental design and analyses see section **4.2.3.1**.

Table 4.10 The drought barley pot trial (Trial 3B) stem length and stem dry weight (DW) in large and small tillers following INCYDE, TDZ-K and CPPU treatment.

Treatment	Large Stem Length (mm)	Small Stem Length (mm)	Large Stem DW (g)	Small Stem DW (g)
Control	501.5 ± 3.0	473.4 ± 2.8	0.5 ± 0.02	0.4 ± 0.02
INCYDE 50 µM	532.8 ± 18.6	483.6 ± 10.8	0.5 ± 0.02	0.4 ± 0.03
TDZ-K 50 µM	523.7 ± 21.1	482.2 ± 10.2	0.5 ± 0.05	0.4 ± 0.03
CPPU 100 µM	524.9 ± 14.3	469.5 ± 6.1	0.4 ± 0.02	0.3 ± 0.02
Treatment *F*-value ($F_{3, 6}$)[a]	0.7	1.05	1.98	2.94
Treatment *p*-value	0.586	0.435	0.219	0.121
Statistics	**Appendix 4.6.1**	**Appendix 4.6.2**	**Appendix 4.6.3**	**Appendix 4.6.4**
Analysis notes	ANOVA	ANOVA	ANOVA	ANOVA

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

Trial 3B was glasshouse pot trial carried out with barley cultivar Tavern over the 2014/15 season. The pot trial was nitrogen-limited and water-limited. The overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates (n = 3), within separate blocks, arranged in a randomised complete block trial (see section **4.2.3** and **Figure 4.2**). For each treatment level replicate, eight whole plants were acquired (24 in total for a treatment level) and plants divided into large and small tillers as described in section **4.2.3.1**. See **Table 4.3** for a summary of the subsample numbers for each trait measured. The control was produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **4.2.1.4**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA and residual plots for the ANOVA assumptions are provided in **Appendices 4.6.1** to **4.6.4** for each trait, and *F*-values and *p*-values are provided. For more information on the experimental design and analyses see section **4.2.3.1**.

Table 4.11 The drought barley pot trial (Trial 3B) thousand grain weight (TGW) and the number of grains per head in large and small tillers following INCYDE, TDZ-K and CPPU treatment.

Treatment	Large TGW Overall (g)	Small TGW Overall (g)	Large no. Grains per Head	Small no. Grains per Head
Control	44.0 ± 1.0	39.8 ± 1.8	6.9 ± 0.5	3.4 ± 0.4
INCYDE 50 µM	45.7 ± 1.1	42.3 ± 0.5	5.1 ± 0.1	3.6 ± 0.8
TDZ-K 50 µM	43.4 ± 0.8	43.2 ± 1.0	5.0 ± 0.1	2.6 ± 0.8
CPPU 100 µM	**39.7 ± 0.2***	**34.5 ± 2.4***	6.5 ± 1.9	2.9 ± 0.7
Treatment *F*-value ($F_{3, 6}$)[a]	7.59	16.2		
Treatment *p*-value	**0.018**	**0.003**	0.707	0.899
Statistics	**Appendix 4.6.5**	**Appendix 4.6.6**	**Appendix 4.6.7**	**Appendix 4.6.8**
Analysis notes	ANOVA, Dunnett test, Levene's *p*-value: 0.571	ANOVA, Dunnett test, Levene's *p*-value: 0.783	Poisson Regression	Poisson Regression

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).
Trial 3B was glasshouse pot trial carried out with barley cultivar Tavern over the 2014/15 season. The pot trial was nitrogen-limited and water-limited. The overall means for treatment levels are described with the standard error. The overall means for treatment levels were generated from three independent replicates (n = 3), within separate blocks, arranged in a randomised complete block trial (see section **4.2.3** and **Figure 4.2**). For each treatment level replicate, eight whole plants were acquired (24 in total for a treatment level) divided into large and small tillers, and ten grains (30 in total for a treatment level) were randomly acquired at different positions as described in section **4.2.3.1**. See **Table 4.3** for a summary of the subsample numbers for each trait measured. The control was produced using a mixture of DMSO (at volumes equivalent to that used by the highest concentration used in treatments) as described in section **4.2.1.4**. Growth stage is represented with GS, and indicates Zadoks scale for cereals (Zadoks *et al*., 1974). Tests including ANOVA, Dunnett test and residual plots for the ANOVA assumptions are provided in **Appendices 4.6.5** to **4.6.8** for each trait, and *F*-values and *p*-values are provided. For more information on the experimental design and analyses see section **4.2.3.1**.

4.3.4 The effect of treatments on chlorophyll content in wheat

4.3.4.1 Wheat GS 39

The effect of PGRs targeted at different growth stages on chlorophyll content in wheat was determined. Following 25 µM INCYDE targeted at wheat at GS 39 (**Table 4.12**), for the flag leaf, there is a statistically significant difference for the time factor ($F_{4, 27}$ = 143.73, p < 0.001, Appendix 4.7.1), with significant loss of chlorophyll at 2 weeks compared to week 0, a significant loss at 4 and 6 weeks compared to all the previous time points and a significant loss of chlorophyll at 8 weeks in comparison to all the previous time points (*post hoc* Tukey HSD, confidence interval: 95%).

In the primary leaves (**Table 4.12**), there is a statistically significant difference in the content of chlorophyll between leaves in the treatment factor ($F_{1, 21}$ = 5.64, p = 0.027, Appendix 4.7.2) with the INCYDE-treated plants having a lower chlorophyll content compared to the control plants (pairwise comparison of control vs. INCYDE, *post hoc* Tukey HSD, confidence interval: 95%). Pairwise comparisons with a *post hoc* Tukey HSD, did not show that there was a significant difference between the INCYDE treatment and control at a specific time point. There is also a statistically significant difference in the content of chlorophyll at different time points ($F_{3, 21}$ = 155.83, p < 0.001), with a significant loss of chlorophyll content at each time point after week 0 compared to all the previous time points (*post hoc* Tukey HSD, confidence interval: 95%).

For the secondary leaves (**Table 4.12**), there is only a significant difference in chlorophyll content with the time factor ($F_{3, 21}$ = 87.23, p < 0.001, Appendix 4.7.3), with a statistically significant loss of chlorophyll at 2 weeks compared to week 0, and a loss at 4 and 6 weeks compared to the earlier time points (*post hoc* Tukey HSD, confidence interval: 95%).

4.3.4.2 Wheat GS 51

With wheat plants targeted with INCYDE and CPPU at GS 51 (**Table 4.13**), with flag leaves, ANOVA indicated an interaction effect, where the impact of treatment on the chlorophyll content depends on the time point that leaves were acquired ($F_{6, 33}$ = 10.25, p < 0.001, Appendix 4.7.4). The interaction plot (Appendix 4.7.4) suggested that there was a disordinal interaction between each treatment and the control. In the interaction plot, INCYDE treatment resulted in a loss of chlorophyll at 2 weeks compared to the control and then chlorophyll levels remained at similar levels to the control from 4 to 6 weeks. In the interaction plot, the CPPU treatment, crossed over the control

between 2 and 4 weeks, with a lower content present in CPPU at 4 weeks compared to the control. Pairwise comparisons of the factors and interaction with a *post hoc* Tukey HSD (confidence interval: 95%) suggested that the effect of INCYDE at 2 weeks resulted in a statistically significant loss in the content of chlorophyll compared to the control at this time point.

In the primary leaves (**Table 4.13**), only the time factor had an effect on the chlorophyll content ($F_{3, 33}$ = 208.98, $p < 0.001$, Appendix 4.7.5), with a statistically significant loss in the content of the primary leaf at each time point after week 0 compared to all previous time points (*post hoc* Tukey HSD, confidence interval: 95%). Likewise, with the secondary leaves, only the time factor had an effect on chlorophyll content ($F_{2, 24}$ = 45.77, $p < 0.001$, Appendix. 4.7.6), with a statistically significant loss in chlorophyll content at each time point after week 0 compared to all previous time points.

4.3.4.3 Wheat GS 61

Following PGRs targeted at wheat at GS 61 (**Table 4.14**), for flag leaves there appeared to be an interaction effect between the treatment factor and time factor ($F_{10, 51}$ = 2.12, $p = 0.039$, Appendix 4.7.7). The interaction plot (Appendix. 4.7.7) suggested that while PGR treatments appeared parallel the control, there was a small crossover of the control means with the INCYDE (25 and 50 µM) means between 4 and 6 weeks after treatment, with the INCYDE treated groups having more chlorophyll at this time point. This suggested an effect on chlorophyll content by the treatment levels INCYDE 25 and 50 µM depended on the time point being 6 weeks after treatment. However, there is no statistically significant difference between the INCYDE (or other) treatments and control at this time point (*post hoc* Tukey HSD, confidence interval: 95%).

For primary leaves (**Table 4.14**), the only effect on chlorophyll content appears to be as a result of the time factor ($F_{1, 33}$ = 1150.52 $p < 0.001$, Appendix 4.7.8), where a statistically significant loss (*post hoc* Tukey HSD, confidence interval: 95%) in the content of chlorophyll is evident at 4 weeks compared to week 0.

Table 4.12 The effect of INCYDE on the content of chlorophyll in flag, primary and secondary leaves of wheat cultivar Morph (Trial 4A) at 0, 2, 4, 6 and 8 weeks following the end of the treatment application (GS 39). INCYDE was applied at 25 µM (GS 39).

Treatment targeted at GS 39		Chlorophyll content (mg/g) weeks after treatment (GS 39)					
Leaf sample	Treatments	Week 0	2 weeks	4 weeks	6 weeks	8 weeks	Analysis notes
Flag	Control	6.4 ± 0.3	4.5 ± 0.3	3.1 ± 0.4	2.5 ± 0.2	0.1 ± 0.02	ANOVA, Tukey HSD
	INCYDE 25 µM	6.3 ± 0.2	4.9 ± 0.4	3.1 ± 0.1	2.9 ± 0.4	0.05 ± 0.01	
	ANOVA	Time ($F_{4, 27}$)[a]	Treatment ($F_{1, 27}$)[a]	Time*Treatment ($F_{4, 27}$)[a]			
	F-value	143.73	0.67	0.41			
	p-value	< **0.001**	0.421	0.799			
	Statistics	**Appendix 4.7.1**					
Primary	Control	8.6 ± 0.7	5.3 ± 0.4	2.3 ± 0.2	0.1 ± 0.1	ANOVA, Tukey HSD	
	INCYDE 25 µM**	7.9 ± 0.5	3.6 ± 0.2	1.7 ± 0.3	0.6 ± 0.3		
	ANOVA	Time ($F_{3, 21}$)[a]	Treatment ($F_{1, 21}$)[a]	Time*Treatment ($F_{3, 21}$)[a]			
	F-value	155.83	5.64	2.37			
	p-value	< **0.001**	**0.027**	0.100			
	Statistics	**Appendix 4.7.2**					
Secondary	Control	7.3 ± 0.7	5.5 ± 0.8	0.8 ± 0.3	0.1 ± 0.004	ANOVA, Tukey HSD	
	INCYDE 25 µM	7.1 ± 0.9	5.8 ± 0.8	0.3 ± 0.1	0.1 ± 0.02		
	ANOVA	Time ($F_{3, 21}$)[a]	Treatment ($F_{1, 21}$)[a]	Time*Treatment ($F_{3, 21}$)[a]			
	F-value	87.23	0.13	0.19			
	p-value	< **0.001**	0.723	0.899			
	Statistics	**Appendix 4.7.3**					

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).
** Indicates a statistically significant difference (*post hoc* Tukey HSD, confidence interval: 95%) of the treatment compared to the control.
The values provided are the overall mean chlorophyll content (mg/g) for four independent replicates (n = 4) for Trial 4A wheat cultivar cultivar Morph (2015/16) following treatment at GS 39 (Zadoks scale for cereals), within separate blocks, arranged in a randomised complete block trial. For each time point, from a single replicate for a treatment level, a subsample of five plants (20 in total for a treatment level) were acquired. Week 0 is defined as the first time point taken after treatment at GS 39. Analyses were carried out at the University of Canterbury by taking flag, primary and secondary leaves from the large/main stems and analysing the leaves according to Evans *et al*. (2012). More details are provided in section **4.2.3.2**. ANOVA analyses were carried out by partitioning for the treatment factor, time factor, block factor and the time*treatment interaction. *F*-values and *p*-values are provided for the ANOVA analyses, which are described in **Appendices 4.7.1** to **4.7.3**. A *post hoc* Tukey HSD (confidence interval: 95%) was carried out when $p \leq 0.05$ for any of the factors of interest (treatment, time or interaction).

Table 4.13 The effect of INCYDE on the content of chlorophyll in flag, primary and secondary leaves of wheat cultivar Morph (Trial 4A) at 0, 2, 4 and 6 weeks following the end of the treatment application (GS 51). INCYDE was applied at 25 µM (GS 51) and CPPU at 100 µM (GS 51).

Treatment targeted at GS 51		Chlorophyll content (mg/g) weeks after treatment (GS 51)				
Leaf sample	Treatments	Week 0	2 weeks	4 weeks	6 weeks	Analysis notes
Flag	Control	5.1 ± 0.5	5.1 ± 0.4	4.7 ± 0.2	0.4 ± 0.2	ANOVA, Tukey HSD, interaction plots
	INCYDE 25 µM	5.7 ± 0.1	**3.3 ± 0.2***	5.2 ± 0.4	0.2 ± 0.1	
	CPPU 100 µM	6.2 ± 0.3	5.8 ± 0.3	3.8 ± 0.2	0.2 ± 0.1	
	ANOVA	Time ($F_{3, 33}$)[a]	Treatment ($F_{2, 33}$)[a]	Time*Treatment ($F_{6, 33}$)[a]		
	F-value	234.42	2.31	10.25		
	p-value	< **0.001**	0.115	< **0.001**		
	Statistics	**Appendix 4.7.4**				
Primary	Control	7.4 ± 0.3	5.1 ± 0.3	2.1 ± 0.7	0.03 ± 0.01	ANOVA, Tukey HSD
	INCYDE 25 µM	6.6 ± 0.6	5.6 ± 0.3	1.3 ± 0.4	0.04 ± 0.01	
	CPPU 100 µM	8.0 ± 0.8	6.1 ± 0.2	1.4 ± 0.4	0.04 ± 0.02	
	ANOVA	Time ($F_{3, 33}$)[a]	Treatment ($F_{2, 33}$)[a]	Time*Treatment ($F_{6, 33}$)[a]		
	F-value	208.98	1.56	1.34		
	p-value	< **0.001**	0.224	0.266		
	Statistics	**Appendix 4.7.5**				
Secondary	Control	7.0 ± 1.1	1.6 ± 0.7	1.5 ± 1.1		ANOVA, Tukey HSD
	INCYDE 25 µM	8.5 ± 0.3	4.2 ± 1.3	0.1 ± 0.03		
	CPPU 100 µM	9.4 ± 0.9	3.4 ± 1.6	0.8 ± 0.7		
	ANOVA	Time ($F_{2, 24}$)[a]	Treatment ($F_{2, 24}$)[a]	Time*Treatment ($F_{4, 24}$)[a]		
	F-value	45.77	1.12	1.27		
	p-value	< **0.001**	0.344	0.308		
	Statistics	**Appendix 4.7.6**				

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

* Indicates a statistically significant difference (post hoc Tukey HSD, confidence interval: 95%) for a treatment compared to the control at a specific time point.

The values provided are the overall mean chlorophyll content (mg/g) for four independent replicates (n = 4) for Trial 4A wheat cultivar cultivar Morph (2015/16) following treatment at GS 51 (Zadoks scale for cereals), within separate blocks, arranged in a randomised complete block trial. For each time point, from a single replicate for a treatment level, a subsample of five plants (20 in total for a treatment level) were acquired. Week 0 is defined as the first time point taken after treatment at GS 51. Analyses were carried out at the University of Canterbury by taking flag, primary and secondary leaves from the large/main stems and analysing the leaves according to Evans *et al*. (2012). More details are provided in section **4.2.3.2**. ANOVA analyses were carried out by partitioning for the treatment factor, time factor, block factor and the time*treatment interaction. *F*-values and *p*-values are provided for the ANOVA analyses, which are described in **Appendices 4.7.4** to **4.7.6**. A *post hoc* Tukey HSD (confidence interval: 95%) was carried out when $p \leq 0.05$ for any of the factors of interest (treatment, time or interaction). An interaction plot was described in the Appendix when ANOVA revealed an interaction effect ($p \leq 0.05$).

Table 4.14 The effect of INCYDE, TDZ-K and CPPU on the content of chlorophyll in flag and primary leaves of wheat cultivar Morph (Trial 4A) at 0, 4 and 6 weeks following the end of the treatment application (GS 61). INCYDE was applied at 25 and 50 µM (GS 61), TDZ-K at 25 and 50 µM (GS 61) and CPPU at 100 µM (GS 61).

Treatment targeted at GS 61		Chlorophyll content (mg/g) weeks after treatment (GS 61)			
Leaf sample	Treatments	Week 0	4 weeks	6 weeks	Analysis notes
Flag	Control	5.4 ± 0.4	2.2 ± 0.4	0.1 ± 0.02	ANOVA, Tukey HSD, interaction plot
	INCYDE 25 µM	5.5 ± 0.6	0.8 ± 0.2	0.4 ± 0.3	
	INCYDE 50 µM	4.5 ± 0.2	1.7 ± 0.1	1.0 ± 0.8	
	TDZ-K 25 µM	4.5 ± 0.4	2.1 ± 0.4	0.04 ± 0.01	
	TDZ-K 50 µM	4.7 ± 0.3	2.0 ± 0.6	0.03 ± 0.003	
	CPPU 100 µM	5.2 ± 0.2	1.1 ± 0.1	0.05 ± 0.001	
	ANOVA	Time ($F_{2, 51}$)[a]	Treatment ($F_{5, 51}$)[a]	Time*Treatment ($F_{10, 51}$)[a]	
	F-value	246.35	0.59	2.12	
	p-value	**< 0.001**	0.711	**0.039**	
	Statistics	**Appendix 4.7.7**			
Primary	Control	6.0 ± 0.7	0.1 ± 0.04		ANOVA, Tukey HSD, interaction plot
	INCYDE 25 µM	6.7 ± 0.6	0.1 ± 0.01		
	INCYDE 50 µM	7.0 ± 0.5	0.1 ± 0.01		
	TDZ-K 25 µM	6.3 ± 0.5	0.1 ± 0.01		
	TDZ-K 50 µM	6.8 ± 0.2	0.7 ± 0.3		
	CPPU 100 µM	6.8 ± 0.3	0.1 ± 0.01		
	ANOVA	Time ($F_{1, 33}$)[a]	Treatment ($F_{5, 33}$)[a]	Time*Treatment ($F_{5, 33}$)[a]	
	F-value	1150.52	1.23	0.77	
	p-value	**< 0.001**	0.316	0.578	
	Statistics	**Appendix 4.7.8**			

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error). The values provided are the overall mean chlorophyll content (mg/g) for four independent replicates (n = 4) for Trial 4A wheat cultivar Morph (2015/16) following treatment at GS 61 (Zadoks scale for cereals), within separate blocks, arranged in a randomised complete block trial. For each time point, from a single replicate for a treatment level, a subsample of five plants (20 in total for a treatment level) were acquired. Week 0 is defined as the first time point taken after treatment at GS 61. Analyses were carried out at the University of Canterbury by taking flag and primary leaves from the large/main stems and analysing the leaves according to Evans *et al*. (2012). More details are provided in section **4.2.3.2**. ANOVA analyses were carried out by partitioning for the treatment factor, time factor, block factor and the time*treatment interaction. *F*-values and *p*-values are provided for the ANOVA analyses, which are described in **Appendices 4.7.7** to **4.7.8**. A *post hoc* Tukey HSD (confidence interval: 95%) was carried out when $p \leq 0.05$ for any of the factors of interest (treatment, time or interaction). An interaction plot was described in the Appendix when ANOVA revealed an interaction effect ($p \leq 0.05$).

4.3.5 The effect of treatments on chlorophyll content in barley

4.3.5.1 Barley GS 51

The effect of PGRs targeted at different growth stages on chlorophyll content in barley was determined. When INCYDE was targeted at barley plants at GS 51 (**Table 4.15**), with flag leaves there was an interaction effect between the treatment and time factors ($F_{2, 15} = 5.06$, $p = 0.021$, Appendix 4.8.1). The interaction plot (Appendix 4.8.1) suggested this interaction was disordinal with INCYDE treatment resulting in an increase in the retention of chlorophyll at 4 weeks. Pairwise comparisons, however, suggested that there is no statistically significant difference between INCYDE and the control at 4 weeks (*post hoc* Tukey HSD, confidence: 95%), or at any other time point.

In the primary leaves (**Table 4.15**), there was also an interaction effect between the treatment and time factors ($F_{1, 9} = 46.22$, $p < 0.001$, Appendix 4.8.2) and an interaction plot (Appendix 4.8.2) indicates that this interaction was disordinal, with INCYDE having an effect on chlorophyll content at 4 weeks. Given the interaction is disordinal, it is not appropriate to attempt to interpret the main treatment effect by itself. Pairwise comparisons showed that there is a statistically significant difference between the control and INCYDE at 4 weeks (*post hoc* Tukey HSD, confidence: 95%), with a significant retention of chlorophyll following INCYDE treatment.

4.3.5.2 Barley GS 61

In flag leaves of barley plants treated with INCYDE and TDZ-K at GS 61 (**Table 4.16**), only the time factor appeared to have an effect on the content of chlorophyll ($F_{3, 45} = 180.51$, $p < 0.001$, Appendix 4.8.3), with each time point after week 0 having a statistically significant loss of chlorophyll compared to all the preceding time points (*post hoc* Tukey HSD, confidence: 95%). With primary leaves of barley plants targeted at GS 61, there was an interaction effect present ($F_{6, 33} = 3.28$, $p = 0.012$, Appendix 4.8.4), with an interaction plot suggesting that each of the INCYDE and TDZ-K treatments resulted in a greater retention of chlorophyll at 2 weeks compared to the control. Pairwise comparisons of the interaction showed that there was a significant retention of chlorophyll at 2 weeks following 50 µM INCYDE treatment compared to the control (*post hoc* Tukey HSD, confidence: 95%). With secondary leaves, only the time factor had an effect on the content of chlorophyll ($F_{1, 21} = 35.01$, $p < 0.001$, Appendix 4.8.5), with a statistically significant loss of chlorophyll at 2 weeks compared to week 0 (*post hoc* Tukey HSD, confidence: 95%).

Table 4.15 The effect of INCYDE on the content of chlorophyll in flag and primary leaves of barley cultivar Fairview (Trial 4B) at 0, 4 and 6 weeks following the end of the treatment application (GS 51). INCYDE was applied at 25 µM (GS 51).

Treatment targeted at GS 51		Chlorophyll content (mg/g) weeks after treatment (GS 51)			
Leaf sample	Treatments	Week 0	4 weeks	6 weeks	Analysis notes
Flag	Control	4.5 ± 0.6	4.1 ± 0.5	0.4 ± 0.3	ANOVA, Tukey HSD, interaction plot
	INCYDE 25 µM	3.4 ± 0.4	6.0 ± 0.8	0.5 ± 0.2	
	ANOVA	Time ($F_{2, 15}$)[a]	Treatment ($F_{1, 15}$)[a]	Time*Treatment ($F_{2, 15}$)[a]	
	F-value	47.76	0.57	5.06	
	p-value	**< 0.001**	0.464	**0.021**	
	Statistics	**Appendix 4.8.1**			
Primary	Control	4.2 ± 0.7	1.6 ± 0.5		ANOVA, Tukey HSD, interaction plot
	INCYDE 25 µM**	3.4 ± 0.4	**6.0 ± 0.6***		
	ANOVA	Time ($F_{1, 9}$)[a]	Treatment ($F_{1, 9}$)[a]	Time*Treatment ($F_{1, 9}$)[a]	
	F-value	< 0.01	20.90	46.22	
	p-value	0.946	**0.001**	**< 0.001**	
	Statistics	**Appendix 4.8.2**			

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).
* Indicates a statistically significant difference (post hoc Tukey HSD, confidence interval: 95%) for a treatment compared to the control at a specific time point.
** Indicates a statistically significant difference (post hoc Tukey HSD, confidence interval: 95%) of the treatment compared to the control.
The values provided are the overall mean chlorophyll content (mg/g) for four independent replicates (n = 4) for Trial 4B barley cultivar Fairview (2015/16) following treatment at GS 51 (Zadoks scale for cereals), within separate blocks, arranged in a randomised complete block trial. For each time point, from a single replicate, for a treatment level a subsample of five plants (20 in total for a treatment level) were acquired. Week 0 is defined as the first time point taken after treatment at GS 51. Analyses were carried out at the University of Canterbury by taking flag and primary leaves from the large/main stems and analysing the leaves according to Evans *et al.* (2012). More details are provided in section **4.2.3.2**. ANOVA analyses were carried out by partitioning for the treatment factor, time factor, block factor and the time*treatment interaction. *F*-values and *p*-values are provided for the ANOVA analyses, which are described in **Appendices 4.8.1** to **4.8.2**. A *post hoc* Tukey HSD (confidence interval: 95%) was carried out when $p \leq 0.05$ for any of the factors of interest (treatment, time or interaction). An interaction plot was described in the Appendix when ANOVA revealed an interaction effect ($p \leq 0.05$).

Table 4.16 The effect of INCYDE and TDZ-K on the content of chlorophyll in flag, primary and secondary leaves of barley cultivar Fairview (Trial 4B) at 0, 2, 4 and 6 weeks following the end of the treatment application (GS 61). INCYDE was applied at 25 and 50 µM (GS 61) and TDZ-K at 25 µM (GS 61).

Treatment targeted at GS 61		Chlorophyll content (mg/g) weeks after treatment (GS 61)				
Leaf sample	Treatments	Week 0	2 weeks	4 weeks	6 weeks	Analysis notes
Flag	Control	8.1 ± 0.2	4.7 ± 0.7	2.3 ± 0.6	0.3 ± 0.2	ANOVA, Tukey HSD
	INCYDE 25 µM	8.5 ± 0.6	5.7 ± 1.1	2.0 ± 0.3	0.3 ± 0.2	
	INCYDE 50 µM	9.0 ± 0.9	3.6 ± 0.6	2.0 ± 0.2	0.1 ± 0.03	
	TDZ-K 50 µM	7.2 ± 0.7	5.3 ± 0.4	2.6 ± 0.2	0.2 ± 0.04	
	ANOVA	Time ($F_{3, 45}$)[a]	Treatment ($F_{3, 45}$)[a]	Time*Treatment ($F_{9, 45}$)[a]		
	F-value	180.51	0.47	1.74		
	p-value	< **0.001**	0.702	0.106		
	Statistics	**Appendix 4.8.3**				
Primary	Control	8.4 ± 0.7	1.6 ± 0.3	1.2 ± 0.2		ANOVA, Tukey HSD, interaction plot
	INCYDE 25 µM	8.9 ± 0.2	3.7 ± 0.4	1.0 ± 0.2		
	INCYDE 50 µM	8.2 ± 0.6	**4.4 ± 0.3***	1.3 ± 0.3		
	TDZ-K 50 µM	7.2 ± 1.1	3.0 ± 0.2	1.7 ± 0.3		
	ANOVA	Time ($F_{2, 33}$)[a]	Treatment ($F_{3, 33}$)[a]	Time*Treatment ($F_{6, 33}$)[a]		
	F-value	230.90	2.44	3.28		
	p-value	< **0.001**	0.082	**0.012**		
	Statistics	**Appendix 4.8.4**				
Secondary	Control	8.3 ± 0.8	2.5 ± 1.0			ANOVA, Tukey HSD
	INCYDE 25 µM	9.2 ± 2.1	3.6 ± 0.8			
	INCYDE 50 µM	10.1 ± 2.3	3.5 ± 0.3			
	TDZ-K 50 µM	9.4 ± 1.0	5.8 ± 0.6			
	ANOVA	Time ($F_{1, 21}$)[a]	Treatment ($F_{3, 21}$)[a]	Time*Treatment ($F_{3, 21}$)[a]		
	F-value	35.01	0.96	0.49		
	p-value	< **0.001**	0.430	0.693		
	Statistics	**Appendix 4.8.5**				

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

* Indicates a statistically significant difference (post hoc Tukey HSD, confidence interval: 95%) for a treatment compared to the control at a specific time point.

The values provided are the overall mean chlorophyll content (mg/g) for four independent replicates (n = 4) for Trial 4B barley cultivar Fairview (2015/16) following treatment at GS 61 (Zadoks scale for cereals), within separate blocks, arranged in a randomised complete block trial. For each time point, from a single replicate for a treatment level, a subsample of five plants (20 in total for a treatment level) were acquired. Week 0 is defined as the first time point taken after treatment at GS 61. Analyses were carried out at the University of Canterbury by taking flag, primary and secondary leaves from the large/main stems and analysing the leaves according to Evans *et al*. (2012). More details are provided in section **4.2.3.2**. ANOVA analyses were carried out by partitioning for the treatment factor, time factor, block factor and the time*treatment interaction. *F*-values and *p*-values are provided for the ANOVA analyses, which are described in **Appendices 4.8.3** to **4.8.5**. A *post hoc* Tukey HSD (confidence interval: 95%) was carried out when $p \leq 0.05$ for any of the factors of interest (treatment, time or interaction). An interaction plot was described in the Appendix when ANOVA revealed an interaction effect ($p \leq 0.05$).

4.4 Discussion

4.4.1 Barley pot trials

4.4.1.1 Senescence

There is no evidence that TDZ-K application results in an increase in the retention of chlorophyll when targeted at anthesis in wheat (**Table 4.14**) or barley (**Table 4.16**). This observation contradicts the reported capacity of TDZ-K being able delay senescence in wheat and barley in detached leaf assays (at concentration ranges between 0.1 to 100 µM) (J. Nisler, personal communication, August 28, 2017; United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript). It is possible that a lack of an observed effect was a result of targeting anthesis, rather than earlier growth stages.

In contrast, in barley, INCYDE application was observed to enhance the retention of chlorophyll in barley when targeted at GS 51 (**Table 4.15**) and GS 61 (**Table 4.16**). The delay of senescence following INCYDE treatment suggests that an enhancement in cytokinin, following inhibition of CKX, is responsible for the retention in chlorophyll. This cytokinin-induced delay of senescence aligns with research where chlorophyll was preserved following exogenous application (Noodén *et al*., 1997) or ectopic *IPT*-overexpression (Guo and Gan, 2014, and references therein). The absence of an enhancement in chlorophyll in the barley field trial (**Table 3.16**), in contrast, suggests that the senescence inhibiting effect of INCYDE depended on the presence of a sub-optimal environment which was provided by the pot trials.

4.4.1.2 Yield

The retention of chlorophyll in barley following treatment INCYDE treatment (**Table 4.15** and **4.16**) did not correspond with an increase in the TGW, which might have been expected from a prolonged photosynthesis in the leaves which should allow for an increase in production of photosynthates, and a subsequent enhancement in yield (Gan and Amasino, 1995). Instead it resulted in no change in yield under nitrogen-limited (**Table 4.7**) or drought trials (**Table 4.11**),but corresponded with a reduction in the head DW in nitrogen-limited conditions (**Table 4.7**). This enhancement of chlorophyll in barley could potentially correspond with the reduction in TGW in barley field trials (**Table 3.13**). As discussed with the field trials (Chapter 3), there is evidence with rice that there is a decrease in yield corresponding with slow senescing traits (Jiang *et al*., 2004; Rubia *et al*., 2014, and references

therein), due to a delay in senescence retarding photosynthate remobilisation, leading to a reduction in grain filling (Zhang *et al*., 1998; Rubia *et al*., 2014).

4.4.2 Wheat pot trials

4.4.2.1 INCYDE

In contrast to barley, in wheat the application of INCYDE at GS 39 (**Table 4.12**) and GS 51 (**Table 4.13**) resulted in a decrease in the content of chlorophyll in the upper leaves (flag and primary). This decrease could correspond with the decrease in the head DW of small tillers (**Table 4.8**). There could be several reasons for this stark difference with wheat, including differences in the timing and duration of growth stages, and species-specific differences in senescence between wheat and barley (Gregersen *et al*., 2008). These include differences in endogenous cytokinin during development, such as the accumulation of *c*Z early in grain development in barley (Powell *et al*., 2013), and even differences in plant architecture, including the positions of leaves and the canopy provided by upper leaves, which could alter the level of shade over the lower leaves and affect senescence (Gregersen *et al*., 2013).

This contrasting effect of INCYDE on wheat (in comparison to barley) also indicates that control over senescence by cytokinin is complex and tightly regulated, and simply enhancing the content of cytokinin by inhibiting CKX activity might not lead to expected or consistent effects. This is in agreement with findings from autoregulatory transgenic *IPT*-overexpression plants, where cytokinin biosynthesis needed to be modest and fine-tuned with feedback regulated by the levels of cytokinin, otherwise abnormal and inconsistent effects were likely to be observed (Guo and Gan, 2014, and references therein). It was also suggested by Rubia *et al*. (2014), following work with slow and fast-senescing lines, that cytokinin dynamics, rather than just the cytokinin levels, were important for controlling senescence, further indicating that an enhancement of cytokinin might be insufficient.

4.4.2.2 TDZ-K

The decrease in stem diameter of wheat treated with TDZ-K under drought (and nitrogen-limited) (**Table 4.9**) conditions suggests that TDZ-K might have some affect on the growth, but only under these stressed conditions. In contrast to the wheat field trials, where TDZ-K targeted at anthesis resulted in a decline in the content of chlorophyll in primary leaves (**Table 3.14**), no such decline was observed in wheat pot trials (**Table 4.14**). This suggested that the effect or efficacy of TDZ-K was

dependent on whether the environment was optimal (field trials) or sub-optimal/stressed (pot trials). The pot trials were not nitrogen optimised (or optimised by other PGR application) in contrast to the field trials. In environments that are nitrogen-limited, senescence is accelerated (Jordi *et al*., 2000; Gregersen *et al*., 2013), while there is also evidence to suggest that nitrogen deficiency affects photosystem II in several ways, and is able to decrease active photosystem II reaction centres in cereals (Lu *et al*., 2001). These findings might help explain why TDZ-K may differentially affect senescence in pots in comparison to field trials. Ultimately, given the disappointing lack of effect of TDZ-K, future experiments should focus on targeting earlier growth stages, including head emergence in cereals, to determine if TDZ-K affects senescence and/or yield.

4.4.2.3 CPPU

The decline in the TGW overall and at positions 1 and 2 in wheat, following CPPU 30 µM (GS 61, 65) (**Table 4.6**) treatment reflects the observed decline in TGW in the wheat field trials following the same treatment applied at the same growth stage (**Table 3.11**). Interestingly, in field trials and nitrogen-limited trials this decline is not reflected when CPPU was applied at the higher concentration (100 µM), suggesting that this effect was dependent on a specific concentration or narrow concentration range. A decline is, however, observed when CPPU was applied at 100 µM under drought (nitrogen-limited) conditions (**Table 4.11**), and this consistent decline occurred across a range of conditions: optimised field trials, nitrogen-limited pot trials, and drought and nitrogen-limited glasshouse pot trials, all of which indicate a clear effect of CPPU, irrespective of the environment.

There are a number of possible reasons for the decline in yield observed following treatment with CPPU, and some of these reasons were discussed previously in sections **3.4.1** and **3.4.2**. This includes the possibility that PGR uptake was limited by the application method, the pleiotropic and complex nature of cytokinins (Jameson and Song, 2016; Koprna *et al*., 2016) and the fact that cytokinin content needed to be manipulated modestly, in order to have beneficial effects on senescence and yield. If endogenous cytokinin levels are increased beyond normal levels in any organ, this is likely to affect a multitude of other organs via the vasculature and affect the sink-source relations of these organs.

4.4.3 Summary

It was evident from the pot trials that INCYDE has the capacity to enhance the chlorophyll content in barley. This retention did not correspond with any change in grain yield, only a decrease in the DW of the heads. Wheat, in contrast, had a decline in chlorophyll following INCYDE treatment, and several reasons for this contrast are discussed.

TDZ-K appeared to have little effect on the content of chlorophyll or yield under any of the conditions tested. This contrasted with the decrease in chlorophyll measured in the wheat field trials, suggesting its efficacy was environmentally-dependent. More recent evidence with TDZ-K suggested that pre-anthesis growth stage targeting would be more likely to result in an enhancement of yield, and potentially a change in chlorophyll content. CPPU application, in contrast to TDZ-K, caused a decrease in the TGW irrespective of the environment and experimental condition. This consistent decline warrants more investigation.

Ultimately, as was the case with the field trials, most changes in yield were decreases in the TGW, suggesting that in the case of CKX-inhibiting INCYDE and CPPU, the effect might be the manipulation of cytokinin was too imprecise, particularly in comparison to other approaches which have been successful for yield enhancement, including the use of autoregulatory transgenic *IPT*-overexpression plants. The experiments in this Chapter ultimately indicate the difficulty and complexities involved with enhancing yield by applying PGRs that act to manipulate endogenous cytokinin (by inhibiting degradation), and indicate that future research is required.

Chapter 5
Gene expression and LC-MS/MS analyses

5.1 Introduction

In the preceding Chapters, the claims made of the effect of INCYDE, TDZ-K and CPPU, were tested with RCBr in controlled growth rooms, and in field and pot trials with wheat and barley. The application of INCYDE resulted in an enhancement in yield, but only when RCBr plants were grown with 5 mM KNO_3 (**Table 2.4**), and in contrast to previous experiments (unpublished data, Palacký University), it did not affect other traits including growth, other components of yield or floral development.

In field trials, neither INCYDE, TDZ-K nor CPPU were able to enhance yield, with INCYDE and CPPU reducing the TGW when targeted at anthesis (**Tables 3.11** and **3.13**), and TDZ-K reducing the content of chlorophyll in wheat primary leaves (**Table 3.14**). In pot trials, the application of PGRs did not enhance yield, with CPPU decreasing the TGW under nitrogen-limited and drought conditions (**Table 4.6** and **4.11**). In pot trials INCYDE application did, however, enhance chlorophyll content in primary leaves in barley when targeted at GS 51 and 61 (**Table 4.15** and **4.16**), while conversely decreasing chlorophyll content in wheat flag leaves when targeting GS 51 (**Table 4.13**). The lack of yield enhancement following CPPU application in field and pot trials aligns with the previously reported limited success CPPU has had with cereals (Jameson and Song, 2016, and references therein), while INCYDE application was unable to replicate the yield enhancement observed following spraying arabidopsis and winter rapeseed at 10 and 50 µM (unpublished data, Palacký University; personal communication, May 22, 2012) with either optimal field trials or stress pot trials. Field and pot trials did not confirm the purported ability of TDZ-K to delay senescence (using detached wheat and barley leaf assays using concentrations between 0.1 to 100 µM) (United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript).

Although there was a lack of yield enhancement in field trials and the difficulties replicating the results purported in previous experiments for INCYDE and TDZ-K, in order to understand this disparity in efficacy and the lack of effect, an investigation into the effect of each compound on the expression of genes involved in cytokinin metabolite homeostasis and the concentration of different cytokinins was carried out.

There has been considerable progress in understanding the genetic mechanisms underpinning wheat development in the last decade. This has been driven by the release of the wheat genome (https://www.wheatgenome.org/), and recent expression studies focused on investigating genes associated with cytokinin regulation and homeostasis (Song *et al*., 2012). Genes have been identified and studied in wheat that encode for isopentenyltransferases (*IPT*) (Song *et al*., 2010; 2012), cytokinin oxidase/dehydrogenases (*CKX*) (Galuszka *et al*., 2004; Zhang *et al*., 2007; 2011; 2012; Feng *et al*., 2008; Ma *et al*., 2011; Song *et al*., 2010; 2012), zeatin *O*-glucosyltransferase (*TaZOG*) (Song *et al*., 2012), *β*-glucosidases (TaGLU) (Sue *et al*., 2006; Song *et al*., 2012), type A response regulators (*TaRRA*) (Song *et al*., 2012) and cell wall invertases (*CWINV*) (Koonjul *et al*., 2004). Gene expression studies of wheat grains revealed that some *TaIPT*s and *TaCKX*s show very strong expression post-anthesis in grain, particularly *TaCKX1*, *TaCKX2* and *TaIPT2* (Song *et al*., 2012). There is a coinciding increase or high level of endogenous cytokinin post-anthesis in wheat grain (Jameson *et al*., 1982; Dietrich *et al*., 1995; Banowetz *et al*., 1999a; 1999b), and this elevation of cytokinin is important for grain development (Dietrich *et al*., 1995; Banowetz *et al*., 1999b; Brugière *et al*., 2008). In barley, an increase in *c*Z type cytokinin has been observed early in development, with an increase in *t*Z types in later stages (Powell *et al*., 2013). The high levels of *TaCKX* expression in wheat during anthesis, when cytokinin levels are critical for grain development, indicated that anthesis was an important target for CKX-inhibiting INCYDE and CPPU in field and pot trials.

Gene expression work with *Brassica* spp. has been supported by the whole genome sequencing of *B. rapa* (Wang *et al*., 2011a), and subsequent genome sequencing of *B. napus* (Chalhoub *et al*., 2014) and *B. oleracea* (Liu *et al*., 2014). Recent gene expression studies have been carried out with RCBr (O'Keefe *et al*., 2011), Chinese cabbage (Liu *et al*., 2013) and *B. napus* (Song *et al*., 2015). Given there is evidence for high levels of expression of some *CKX*s in young leaves of *Brassica* spp. (O'Keefe *et al*., 2011; Song *et al*., 2015), growth room experiments targeted INCYDE onto young leaves in RCBr (Chapter 2), and led to an enhancement in seed yield (**Table 2.3**).

Using knowledge from the results in preceding Chapters, together with recent gene expression studies (O'Keefe *et al*., 2011; Liu *et al*., 2013; Song *et al*., 2012; 2015) and reports of endogenous cytokinin in cereal grains (Dietrich *et al*., 1995; Banowetz *et al*., 1999b; Brugière *et al*., 2008; Powell *et al*., 2013), an investigation into the effect of INCYDE, TDZ-K and CPPU treatments on cytokinin homeostasis and endogenous levels was carried out.

5.2 Materials and Methods

5.2.1 Sample acquisition and preparation

5.2.1.1 Wheat and barley grains

Wheat (Torch) and barley (Quench) heads were removed from field trials in November and December of 2014/2015 season (Chapter 3). Wheat heads were removed at and following anthesis (GS 61), where 100 µM TDZ-K and 100 µM CPPU was applied to wheat and 50 µM INCYDE was applied to barley plants. Due to first field trial being infected with *Septoria*, grain samples were not acquired from wheat following INCYDE treatment. These concentrations are among the higher concentrations used (with effect) on previous experiments which are described in sections **3.1.2** and **4.1.2**, as the objective was to measure a response, these high concentrations which were more likely to have an effect were selected. Treatment solutions contained 0.5% DMSO and 0.5% Yates Sprayfix (Yates). The experiment was arranged as a randomised complete block design, with three blocks (**Figure 5.1**). Within each replicate for each treatment level, ten whole plants were acquired. Three replicates (made up of 30 plants total) for each treatment level were acquired, and each treatment level was represented once.

Heads were acquired at days 0, 1, 2, 4, 7 and 14 d after anthesis and treatment (daa/t), with the first samples taken within 2 h after the first application. Two applications were made during anthesis (GS 61 and GS 65) in order to ensure heads, which developmentally varied slightly between plants, were sprayed during anthesis. The second application was made between days 4 and 7 following the first application. This marked an extra application of TDZ-K and CPPU between days 4 and 7 following the first GS 61 application on wheat. Wheat and barley heads were immediately flash frozen in liquid nitrogen, transported to the lab and stored at -80°C. Grains were removed from the middle third section of each head and sorted by grain development stages based on Song *et al*. (2012). Growth stages were standardised against the days after PGR treatments were applied, for example, only grain that were observably at a point in development two days after anthesis (daa), were taken from heads that were removed two days after treatment (dat). With each replicate for each treatment the date-adjusted grains were pooled, creating three separate pools (replicates) for each treatment level. Samples were then ground with a mortar and pestel into a fine powder under liquid nitrogen in an RNAase-free environment.

5.2.1.2 Wheat flag leaves

Flag leaves were excised from wheat (Morph) plants grown in pots in the 2015/16 season (Trial 4A, Chapter 4). The experimental design was a randomised complete block design with three blocks. As described for wheat heads, within each replicate for each treatment level, ten whole plant samples were acquired following treatments (**Figure 5.1**). These treatments included 50 µM INCYDE and 50 µM TDZ-K (0.5% DMSO, 0.5% Yates Sprayfix (Yates)) targeted at anthesis. Given the limited space available for carrying out these pot trials, only INCYDE and TDZ-K were applied in this experiment. Flag leaf samples were excised from each plant and immediately flash frozen and stored at -80°C. Samples were taken at 0 d (2 to 3 h prior to treatment) and then 1, 2, 4 and 7 daa/t. As previously described for grains, for each treatment replicate, flag leaf samples were pooled (giving three pools for each treatment level) and then ground into a fine powder under liquid nitrogen.

5.2.1.3 Rapid cycling *Brassica rapa* samples

Rapid cycling *B. rapa* plants were grown in growth rooms, arranged in pots within treatment trays using a randomised complete block design, with three blocks. Each treatment level replicate was represented by a tray containing pots with four plants per pot and each treatment level represented once in each of the three blocks (**Figure 5.1**). Plants were provided with continuous 1 mM KNO_3 (Univar) and thinned out to four to five plants per pot after cotyledons were visible. Plants were maintained as described in section **2.2.1** After the appearance of leaves at 11 days after sowing, single PGR treatments of 50 µM INCYDE or 50 µM TDZ-K (0.5 % DMSO with 0.1% surfactant Silwet L-77 (Agri-Turf Supplies)) were made onto plants until runoff to give a total of 0.5 mL per plant.

Due to the importance that nitrate concentration played in the effect of INCYDE on yield in the growth room experiments (**Table 2.3**), the effect of nitrate on the expression of cytokinin regulatory genes was also investigated in this Chapter. For the nitrogen treatment group, RCBr plants were provided with 10 mM KNO_3 (Univar) (up from 1 mM KNO_3) and continuously supplemented with high levels of nitrate until the end of the RCBr life cycle. This treatment group will be referred to as the 'high N' group throughout this Chapter.

For each replicate for each treatment level, five plants were removed (giving a total of 15 plants for the three replicates for each treatment level). The lower leaves, which were the oldest/first leaves to appear on RCBr plants, were excised as the younger leaves were too small at the time point samples were acquired, and it would be too difficult to extract sufficient quantities of RNA for gene expression analysis. Leaf samples were immediately flash frozen and stored at -80°C. Leaf samples were excised at 0 d (2 h prior to treatment), and then 1, 2, 4, 8 and 16 d after treatment with PGR or nitrate. These time points covered development stages, immediately after leaf appearance and before bud development (0 to 2 days after treatment), just prior to flowering (4 to 8 dat), and mid to late flowering (16 dat). Control groups were sprayed with a water solution containing 0.5% DMSO and 0.1% Silwet L-77, and maintained with a 1 mM KNO_3 treatment solution over the duration of the experiment.

Preliminary growth room experiments were carried out with CPPU and found an effect on flowering, the data is not shown as a full set of replicates was not completed. The effect of CPPU application on the expression of genes during flowering was therefore also investigated in this Chapter. Rapid cycling *Brassica rapa* plants were grown under 5 mM KNO_3 (Univar), and sprayed with a single

application 100 µM CPPU (0.1% ethanol with 0.01% TWEEN® 20 (Sigma-Aldrich)) treatment during flowering, made at 20 d after sowing. Leaf samples were excised at 3, 6 and 24 h following treatment, flash frozen and stored as previously described. For each replicate of a treatment, leaf samples were pooled and ground up into a fine powder to produce three replicates for each treatment level.

RCBr plants were treated with 50 µM INCYDE with surfactant Silwet L-77 (as opposed to 25 µM INCYDE with Tween 20 in Chapter 2) and 1 mM KNO_3 (as opposed to the 5 mM KNO_3 used when yield was enhanced in Chapter 2) as subsequent experiments following Chapter 2 revealed a yield enhancement in plants grown in nitrogen-limiting conditions (0.1-1 mM KNO_3) with INCYDE applied using "super-spreader" Silwet L-77, a surfactant similar to that used in preliminary experiments at Palacký University (unpublished data, Palacký University). These preliminary replicates did not, however, appear to show that INCYDE applied with Silwet L-77 had a broad effect on growth and yield traits as described in the Palacký University results. These yield results were not reported because not all replicates for this experiment were carried out to completion. However, given that this surfactant was similar to that used at Palacký University, it was applied in RCBr experiments that were used for gene expression analyses.

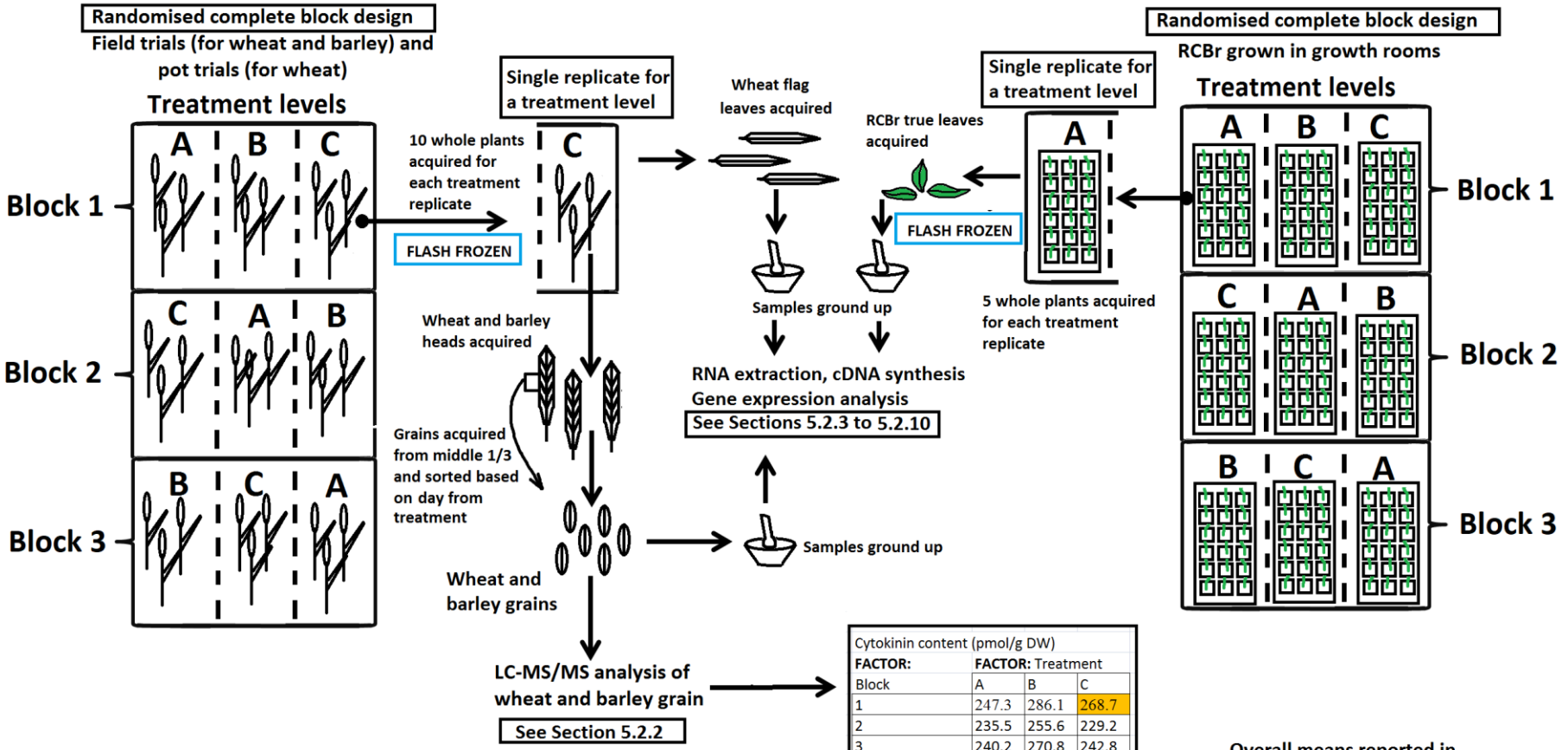

Figure 5.1 Experimental design, and how grain and leaf samples were acquired, ground up and analysed by LC-MS/MS and RT-qPCR. Samples were acquired from field, pot trials and growth room experiments arranged in a randomised complete block design with three blocks.

5.2.2 LC-MS/MS analyses of endogenous cytokinins

Wheat and barley grain samples (section **5.2.1.1)**, at four days following anthesis/treatment application, were ground under liquid nitrogen and freeze dried using a Savant SPD232DDA SpeedVac Concentrator (Thermo Fisher Scientific), to produce 8 to 22 mg of freeze dried ground grain. For each treatment, three replicates from separate blocks were acquired (**Figure 5.1**). These samples were then sent to the Laboratory of Growth Regulators (Palacký University, Olomouc), and analysed using a protocol described in Svačinová *et al*. (2012). Samples were extracted using a modified Bieleski buffer (60% MeOH, 10% HCOOH 30% and H_2O) with stable isotope-labelled cytokinin standards. Samples were analysed by LC-MS/MS using an Acquity UPLC® System (Waters), with a binary solvent and sample manager linked to a 2996 PDA detector (Waters) and triple quadrupole mass spectrometer XevoTM TQ MS (Waters). Mass spectrometry data was processed using MassLynx™ Mass Spectrometry Software and TargetLynx™ (Waters). The concentration of different cytokinins was provided in pmol/g DW.

To determine if there were statistically significant differences between the control and treatment groups in the content of each cytokinin type, an ANOVA (significance level: 0.05, two sided) was carried out using Minitab 17 (Minitab Inc.), looking at the treatment factor and the block factor (Appendices 5.1 and 5.2). All factors were fixed. Where the p-value was ≤ 0.05 for the treatment factor, a *post hoc* two sided Dunnett test (confidence interval: 95%) was carried out using XLSTAT 2016 (Addinsoft) to determine if there was a statistically significant difference between the control and the treatments. The ANOVA assumptions of normal distribution of the residuals and the homoscedasticity of the variances were met by examining the residual plots including a Q-Q plot and a plot of the standardised residuals against predicted values. Where there were significant differences between treatments, a Levene's test *p*-value was also reported. The overall mean concentration of each cytokinin type was calculated using the three replicates and, along with the standard error, reported in the results tables (**Table 5.3** and **5.4**).

5.2.3 RNA extraction

RNA extraction for RT-qPCR analysis was carried out using two different protocols: a TRIzol™ protocol and a RNeasy Plant Mini Kit (Qiagen). The RNeasy Plant Mini Kit protocol was not available when early RNA extractions were made, so the TRIzol™ protocol was used for early experiments. Both protocols were carried out in an RNAase-free room, where every surface and tool

was regularly cleaned with RNaseZAP (Thermo Fisher Scientific). Protocols were also carried out quickly to reduce RNA degradation.

5.2.3.1 TRIzol RNA extraction protocol

The TRIzol™ RNA extraction protocol was used for RCBr leaf material from the CPPU experiment only, as this experiment was carried out early when the RNeasy Plant Mini Kit protocol was not available. Using no more than 200 mg of ground leaf sample in 1.7 mL centrifuge tubes, 1 mL of TRIzol™ reagent (Thermo Fisher Scientific) was added to the sample, vortexed briefly and then left to stand for 3 min at room temperature. The sample was centrifuged (12 000 *g*, 4°C, 2 min), and the supernatant transferred to a new tube. Chloroform (200 µL) was added to the sample, shaken vigorously for 15 s and then left to stand for 5 min at room temperature.

Samples were then centrifuged (12 000 *g*, 4°C, 15 min), the supernatant transferred to a new tube and left to stand at room temperature for 2 min. Isopropanol (500 µL) was added to the sample, mixed carefully, left for 10 min at room temperature and then centrifuged (12 000 *g*, 4°C, 5 min). The supernatant was carefully decanted and the RNA pellet washed twice with 1 mL 75% ethanol. The sample was vortexed to suspend the RNA pellet. Samples were centrifuged (12 000 *g*, 4°C, 2 min), the ethanol was removed and the pellet air dried for 10 min.

The pellet was dissolved in 30 to 50 µL of 1x of RNA secure™ (Thermo Fisher Scientific), and activated by heating at 65°C for 15 min. The sample was mixed by tapping the bottom of the tube every 3 to 5 min. The RNA samples were then stored at -20°C until further use.

5.2.3.2 RNeasy Plant Mini Kit protocol

For all other samples, including RCBr leaves (aside from CPPU experiments), wheat flag leaves and wheat grain samples, RNA was extracted using the RNeasy Plant Mini Kit (Qiagen). Ground grain or leaf sample (20 to 100 mg) was mixed with 450 µL RLC (Qiagen) for grain samples or RLT buffer (Qiagen) for leaf samples, containing 1% β-mercaptoethanol. Following RLT or RLC addition, the samples were immediately vortexed for 30 s. Leaf samples were incubated at 56°C for 1 to 3 min.

The lysate was then transferred to a QIAShredder spin column (Qiagen) in a 2 mL collection tube and centrifuged (18 000 *g*, 2 min). The flow-through supernatant was removed without disturbing the pellet and transferred to a new Eppendorf tube. A half volume of absolute ethanol > 99% (Univar)

was added and mixed carefully by pipetting. The sample was then transferred into a RNeasy spin column (Qiagen) and centrifuged (14 000 *g*, 35 s) and the flow-through discarded. This step was repeated using the same column with a new sample in order to pool samples with low RNA yields. No more than four samples were pooled onto the same column.
On-column DNAase digestion was carried out to remove DNA contamination using the RNase-Free DNase Set (Qiagen). Subsequently, 350 µL of RW1 buffer (Qiagen) was added to the column, samples were centrifuged (14 000 *g*, 35 s) and the flow-through discarded. DNase I (10 µL) (Qiagen) stock solution was added to 70 µL RDD buffer (Qiagen) and mixed gently by tube inversion. DNase I mix (80 µL) was added directly to the RNeasy column membrane, and the samples were left at room temperature for 15 min. The column was washed with 350 µL RW1 (Qiagen) by centrifugation (14 000 *g*, 35 s), and the flow-through discarded.

A RPE buffer (Qiagen) mix was prepared by mixing 500 µL RPE buffer (Qiagen) with four volumes of absolute ethanol (> 99%, Univar) and 500 µL of this RPE-ethanol mix was added to the column and the column centrifuged (14 000 *g*, 35 s). The flow-through was discarded. Another 500 µL of RPE-ethanol mix was added to the column and centrifuged (13 000 *g*, 150 s), with the flow-through once again discarded. The column was centrifuged again (18 000 *g*, 95 s) in a collection tube to dry the column and remove any ethanol carry-over. The column was placed into a new 1.5 mL collection tube and 10 to 45 µL of RNase-free water (Qiagen) was added directly to the column, which was centrifuged (18 000 *g*, 95 s). This centrifugation step was repeated with the eluate. The eluted RNA was stored at -20°C until further use.

5.2.4 Determining RNA integrity, purity and concentration

RNA concentration and purity were assessed using NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific) with software NanoDrop 1000 v 3.8.1. Sample (2 µL) was added to the Nanodrop, and absorbance measured at 260 nm. The concentration of most samples was > 100 ng/µL. Ratios at 260/280 nm and 260/230 nm indicate the purity of the RNA sample, and only samples with ratios > 1.8 for 260/280 and 260/230 were selected as acceptable for producing cDNA.

RNA integrity was determined using 1% (w/v) agarose gel (Appendix 1.1). Samples were mixed with 6x agarose gel loading dye (Appendix 1.1.2), and 2 µL of HyperLadder™ 1 kb (Bioline) was used to determine the size of the bands. Gels were set to 80 V for 25 min. Gel bands were visualised using a UV transilluminator G:BOX (Syngene) with software GeneSys (Syngene). RNA integrity was

determined by the presence of two clear 28S and 18S RNA bands, where it was assessed whether the 28S rRNA was twice as bright as the 18S band. The absence of a 2:1 ratio in intensity of the 28S:18S bands or significant smears indicated RNA degradation or reduced integrity and these samples were also disposed of.

5.2.5 cDNA synthesis

The RNA samples were used for cDNA synthesis. A primer annealing mix (Appendix 1.2.1) was produced by mixing 500 to 1000 ng RNA with 0.5 µL of 25x RNA secure™ (Thermo Fisher Scientific), 1 µL of 100 pmol of Random pd$(N)_6$ (Sigma-Aldrich), 1 µL of 50 pmol Oligo$(dT)_{18}$ primers (Bioline), made up to 10 µL with DEPC-treated water. The 25x of RNA secure™ (Thermo Fisher Scientific) was activated by incubating the mix at 65°C before placing samples on ice for 2 min. Samples were added to a reverse transcriptase mix (Appendix 1.2.2) made up of 4 µL RT buffer (Sigma-Aldrich), 1 µL 20 mM dNTP (Thermo Fisher Scientific), 2 µL DTT (Sigma-Aldrich), 1 µL Expand™ Reverse Transcriptase (Sigma-Aldrich) and 2 µL DEPC-treated water. A negative control (-RT) was made for each sample set by excluding Expand™ Reverse Transcriptase (Sigma-Aldrich) and was used to ensure no background genomic (gDNA) contamination affected the amplification or melting curves. Samples were placed in a Mastercycler® pro (Eppendorf), incubated at room temperature for 10 min, 42ºC for 60 min and at 70ºC for 15 min, before being diluted 10x with UltraPure DNase/RNase-Free Distilled Water (Thermo Fisher Scientific). Subsequently samples were stored at -20ºC until analysis by RT-qPCR.

5.2.6 Bioinformatics and primer design

Wheat primers were acquired from Jason Song (Song *et al*., 2012) (**Table 5.1**) and RCBr primers from David O'Keefe (O'Keefe *et al*., 2011) (**Table 5.2**). Cell wall invertase primers (*TaCWINV*) were designed by using the wheat (*Triticum aestivum*) genome which has been sequenced (https://www.wheatgenome.org/). *TaCWINV* genes were identified using Nucleotide BLAST (Basic Local Alignment Search Tool: https://blast.ncbi.nlm.nih.gov/Blast.cgi) from the database of the National Center for Biotechnology Information (NCBI). Genes were identified for *Triticum aestivum*, and compared with orthologous sequences from related species including rice (*Oryza sativa*), maize (*Zea mays*), barley (*Hordeum vulgare*) and purple false brome (*Brachypodium distachyon*). These sequences were aligned using Clustal X 2.0 software (http://www.clustal.org) and their phylogenetic relationships visualised by producing maximum likelihood (ML) phylogenetic trees with Molecular

Evolutionary Genetics Analysis (MEGA) software version 7.0.26, using a bootstrap with 1000 replicates.

Primers for *CWINV* genes were designed based on an approach described in Song *et al*. (2012). Wheat is hexaploid and contains homoeologous chromosomes in the A, B and D genomes (Akhunov *et al*., 2003). To avoid the time-consuming process of designing primers specific to one genome, degenerate primer pairs were designed within conserved regions shared by family members of closely-related cereals. *TaGLU1a* (**Table 5.1**), was previously designed to target a specific homoeologue (Sue *et al*., 2006; Song *et al*., 2012).

Selected sequences were then copied into Primer Premier 6.23 (PREMIER Biosoft) and forward and reverse primers were designed and optimised according to guidelines provided by Premier Biosoft (http://www.premierbiosoft.com). Primers were ordered from Macrogen Inc. (https://dna.macrogen.com/) and diluted with 1x Tris-EDTA buffer (Thermo Fisher Scientific) to produce working solutions.

Table 5.1 *Triticum aestivum* primers designed and used, the sequence, accession numbers and the designer of each.

Gene	F/R Primer	Primer sequence (5' to 3' end)	Gene full name	Accession no. / Locus	Designed by
TaGAPDH	F	CCACTGTTGATGTTTCTGTTGTTGA	Glyceraldehyde-3-phosphate dehydrogenase	AF251217	J. Song
TaGAPDH	R	CCTGTTGTCACCCTGGAAGTCA	Glyceraldehyde-3-phosphate dehydrogenase	AF251217	J. Song
TaELF1	F	TGGAAGTTCGAGACCACCAAGTAC	Elongation factor 1	M90077	J. Song
TaELF1	R	ATCTTGTTGCAGCAGCAGATCATC	Elongation factor 1	M90077	J. Song
TaIPT2	F	GCCGGGGATGATGGAGTGGTA	Isopentenyltransferase 2	JN128577	J. Song
TaIPT2	R	GCTGGAGGACGAGGCGGAA	Isopentenyltransferase 2	JN128577	J. Song
TaIPT3	F	TGGATGTCGACGAGGCAGTTCT	Isopentenyltransferase 3	JN128578	J. Song
TaIPT3	R	ATGCGCCGGATCTTGCCGA	Isopentenyltransferase 3	JN128578	J. Song
TaIPT5	F	CACAGACAAGCTGCACGAAGGA	Isopentenyltransferase 5	JN128579	J. Song
TaIPT5	R	CGATCGGTCAGCTTGTGTACCAAC	Isopentenyltransferase 5	JN128579	J. Song
TaIPT7	F	GCGGTGCTGGAACGGTACGT	Isopentenyltransferase 7	JN128581	J. Song
TaIPT7	R	TGACTGCACACCAGCCTGCA	Isopentenyltransferase 7	JN128581	J. Song
TaIPT8	F	GACGTGGAGGAGGCGCTCCT	Isopentenyltransferase 8	JN128582	J. Song
TaIPT8	R	ATGCGCCGGATCTTGGACA	Isopentenyltransferase 8	JN128582	J. Song
TaCKX1	F	GGAGGTGGCGCTGGACAAGATC	Cytokinin oxidase/dehydrogenase 1	JN128583	J. Song
TaCKX1	R	GCAGAACCGCAGTATCTTCTGGT	Cytokinin oxidase/dehydrogenase 1	JN128583	J. Song
TaCKX2	F	CCAGAGGAGGAGGAGGTGTTCTAC	Cytokinin oxidase/dehydrogenase 2	JN128584	J. Song
TaCKX2	R	TTGGCCGGACCAAAGTGCTT	Cytokinin oxidase/dehydrogenase 2	JN128584	J. Song
TaCKX3	F	GGAGGGCTTCGCGTTCGTG	Cytokinin oxidase/dehydrogenase 3	JN128585	J. Song
TaCKX3	R	CAGGCCCCGCACGTACTTGA	Cytokinin oxidase/dehydrogenase 3	JN128585	J. Song
TaCKX4	F	TGCTGTCTCGGCTGAGATACATACAG	Cytokinin oxidase/dehydrogenase 4	JN128586	J. Song
TaCKX4	R	TGACGTCCTGTTGTCCCACTTTG	Cytokinin oxidase/dehydrogenase 4	JN128586	J. Song
TaCKX8	F	TGCGCGTGGAGGAGGCTGA	Cytokinin oxidase/dehydrogenase 8	JN128589	J. Song
TaCKX8	R	ACAGTGTAGAATACGTCCTCGCCAG	Cytokinin oxidase/dehydrogenase 8	JN128589	J. Song
TaCKX10	F	GGTAAGGTGGATAAGAGTTCTCTACTT	Cytokinin oxidase/dehydrogenase 10	JN128591	J. Song
TaCKX10	R	ATCTGAGTTGAGATAGTAGTGCATGGA	Cytokinin oxidase/dehydrogenase 10	JN128591	J. Song
TaCKX11	F	AGCAACGTCCTGCAGCTCCAA	Cytokinin oxidase/dehydrogenase 11	JN128592	J. Song
TaCKX11	R	GAGCTGCGGATGGAGTGCTCA	Cytokinin oxidase/dehydrogenase 11	JN128592	J. Song
TaGLU1a	F	AATAACAACGCAACGGTGACAGTTA	β-glucosidase 1a	JN128599	J. Song
TaGLU1a	R	GATCCCGACGACATGCAAACA	β-glucosidase 1a	JN128599	J. Song
TaRRA4	F	TTGAAGGACATTCCAGTGGTGAT	Response regulator 4	JN128606	J. Song
TaRRA4	R	TTGAGCTTCTTCATGTCAGCSA	Response regulator 4	JN128606	J. Song
TaZOG2	F	ACTCACCGAGCAGCTGGTCTCA	Zeatin *O*-glucosyltransferase 2	JN128597	J. Song
TaZOG2	R	CCCTTCACCAGGTCGTTCCTCA	Zeatin *O*-glucosyltransferase 2	JN128597	J. Song
TaCWINV1	F	ACCACTTCCGGCCCATAA	Cell wall invertase 1	AB196522	M. van Voorthuizen
TaCWINV1	R	CCGTACTTGTCGGACTTG	Cell wall invertase 1	AB196522	M. van Voorthuizen
TaCWINV2	F	CGTCCTCATGTGCAGTGA	Cell wall invertase 2	AB196523	M. van Voorthuizen
TaCWINV2	R	TGGATGGGTACACTCTCG	Cell wall invertase 2	AB196523	M. van Voorthuizen

Table 5.2 Rapid cycling *B. rapa* primers designed and used, the sequence, accession numbers and the designer of each.

Gene	F/R Primer	Primer sequence (5' to 3' end)	Gene full name	Accession no. / Locus	Designed by
BrGAPDH	F	GATCCCTTCATCACCACCGAGTA	Glyceraldehyde-3-phosphate dehydrogenase	XM_009119750	J. Song/D. O'Keefe
BrGAPDH	R	GGGGAGCAAGGCAGTTAGTG	Glyceraldehyde-3-phosphate dehydrogenase	XM_009119750	J. Song/D. O'Keefe
BrELF1	F	AGGAGGCTGCTGAGATGAACAA	Elongation factor 1	XM_018654655	J. Song/D. O'Keefe
BrELF1	R	CCATCTTGTTACAGCAGCAAATCA	Elongation factor 1	XM_018654655	J. Song/D. O'Keefe
BrIPT1	F	GGGCATGTTCGAGGAGCTGT	Isopentenyltransferase 1	XM_009129386	J. Song/D. O'Keefe
BrIPT1	R	AACGACGCCGTTGCGTCAACT	Isopentenyltransferase 1	XM_009129386	J. Song/D. O'Keefe
BrIPT2	F	GAKTYATGGGATGYGCAAGTGGTTA	tRNA dimethylallyltransferase 2	XM_009142572	J. Song/D. O'Keefe
BrIPT2	R	AATCGTGCGGTTCTCTTACGATGTA	tRNA dimethylallyltransferase 2	XM_009142572	J. Song/D. O'Keefe
BrIPT3	F	GGGATCGACGGTAAGTCGTGAGT	Isopentenyltransferase 3	XM_013854461	J. Song/D. O'Keefe
BrIPT3	R	CACAATTAAAAGATGCGAGGTATGGTA	Isopentenyltransferase 3	XM_013854461	J. Song/D. O'Keefe
BrCKX3	F	GGTTTCTTGGACGGAYTATTTG	Cytokinin dehydrogenase 3	XM_013814227	J. Song/D. O'Keefe
BrCKX3	R	GRTGTCGTTTKAYCATTGAGRC	Cytokinin dehydrogenase 3	XM_013814227	J. Song/D. O'Keefe
BrCKX6	F	TAAATAGGACGGGACTGCTGGACA	Cytokinin dehydrogenase 6	XM_009118749	J. Song/D. O'Keefe
BrCKX6	R	ACCGCAGCTTCATCTCAGAGACAT	Cytokinin dehydrogenase 6	XM_009118749	J. Song/D. O'Keefe
BrCKX7	F	GGGAAAGACTTCGGTGGCA	Cytokinin dehydrogenase 7	XM_013804977	J. Song/D. O'Keefe
BrCKX7	R	CGCCAAGCCATACTCCGA	Cytokinin dehydrogenase 7	XM_013804977	J. Song/D. O'Keefe

5.2.7 Reference genes

Two reference genes were used for each of the RCBr and wheat RT-qPCR analyses: glyceraldehyde-3-phosphate dehydrogenase (*TaGAPDH* and *BrGAPDH*), and elongation factor 1 (*TaELF1* and *BrELF1*). These reference genes were previously used with wheat (Song *et al*., 2012) and RCBr (O'Keefe *et al*., 2011).

5.2.8 RT-qPCR analyses

RT-qPCR was used to determine the relative gene expression. Reactions were carried out using 2x SYBR green mix (Appendix 1.3.1) for RCBr and KAPA SYBR® FAST qPCR Master Mix (2x) (Sigma-Aldrich) for wheat.

Reaction volumes were made up using 7.5 µL of 2x SYBR or KAPA SYBR® (Sigma-Aldrich), 4.5 µL Milli-Q H_2O, 1 µL of cDNA and 1 µL each of a forward and reverse primer. Each sample used 0.1 mL strip tubes (Qiagen). Reactions were carried out with real-time PCR cycler Rotor-Gene Q (Qiagen) and analysed with Rotor-Gene Series Software 2.3.1 (Qiagen).

Cyclers were set to an initial hold time of 3 min (95°C), then 40 cycles at 95°C for 10 s, 52°C to 64°C (depending on the primer used) for 15 s and 72°C for 15 s. Sometimes a fourth step was set at 80 to 85°C for 15 s to eliminate non-specific products including primer dimers. For each biological replicate, three technical replicates were carried out, that is, a full RT-qPCR cycle repeat three times, using the cDNA solution produced for each biological replicate.

Threshold cycle values (Ct) were taken at the centre of the exponential amplification zone (**Figure 5.2A**), and were set at 2000 fluorescent units. The average relative expression of target genes was normalised using the constitutively expressed reference ('house keeping') genes (González-Verdejo *et al*., 2008; Le Bail *et al*., 2008), with the relative expression ratio method of Pfaffl (2001).

$$R = \frac{\left(E_{target}\right)^{\left(\overline{X}(C)_{target} - \overline{X}(S)_{target}\right)}}{\left(E_{reference}\right)^{\left(\overline{X}(C)_{reference} - \overline{X}(S)_{reference}\right)}}$$

In this equation, $\overline{x}\,(C)_{target}$ and $\overline{x}\,(C)_{reference}$ represent the average Ct values of the control for both the target and reference genes, whereas $\overline{x}\,(S)_{target}$ and $\overline{x}\,(S)_{reference}$ represent the average Ct values of the sample subject for both the target and reference genes. The E represents the PCR efficiency for the target and reference genes.

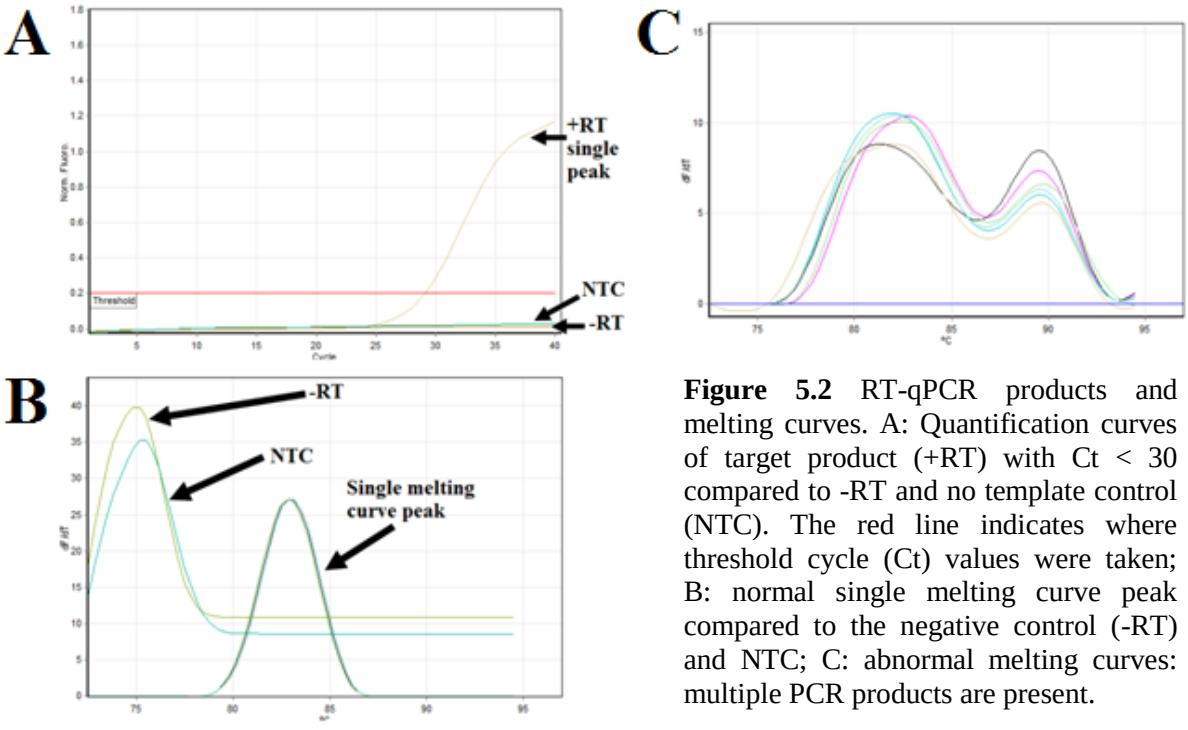

Figure 5.2 RT-qPCR products and melting curves. A: Quantification curves of target product (+RT) with Ct < 30 compared to -RT and no template control (NTC). The red line indicates where threshold cycle (Ct) values were taken; B: normal single melting curve peak compared to the negative control (-RT) and NTC; C: abnormal melting curves: multiple PCR products are present.

5.2.9 Ct calculations and heat map construction

5.2.9.1 Natural expression change

Each biological replicate was made up of samples from each time point taken from a single block. For example, flag leaf samples from 0, 1, 2, 4 and 7 days were taken from the control plot or pots within block 1, and this represented one biological replicate. The expression level over time was calculated according to the method described by Song *et al*. (2012), using two biological replicates from the control (untreated) plants. For a gene of interest, the raw Ct values were recorded for a single biological replicate and these values were corrected using correction factors (CF), which were generated by using Ct values of the reference genes. The expression fold change in control plants was calculated relative to the baseline expression; the lowest level of expression recorded in any of the time points of a single biological replicate. Subtracting the Ct values from the baseline expression gave a delta Ct, which was then used to calculate the fold change by $(2)^n$, where n represents the delta Ct value calculated.

This was repeated with the second replicate. The overall mean for the fold change was generated using the fold change values calculated for each replicate, and the overall means were reported in the results section using a heat map showing the expression change relative to the baseline expression. An example of these heat maps described in the results is shown in **Figure 5.3A**.

5.2.9.2 Treatment expression compared to control expression

The fold change difference between the control and treatments (PGRs and nitrate) was also compared using two biological replicates. Corrected Ct values were calculated for the treatment and control groups as described in section **5.2.9.1**, but in this case, the delta Ct value was calculated by subtracting the Ct at each time point from the Ct value at the first time point (normally 0 d). This sets the first time point as the reference point. The fold change was then calculated using $(2)^n$, where n represents the delta Ct value calculated, and the fold change was reported as a positive or negative value, to show up or downregulation relative to the first time point respectively.

Using the fold change values generated for each replicate, an overall mean fold change was calculated and reported for the control and treatment groups separately in a heat map. In order to compare the treatment and the control expression, for a given time point, the mean fold change for the control was subtracted from the mean fold change of the treatment. This generated a fold change difference, which is displayed in a heat map along with the control and treatment expression change. A visualisation of the heat maps generated that compare the control and treatment groups is described in **Figure 5.3B**.

5.2.10 PCR optimisation

Annealing temperatures were optimised for each primer pair between 52 to 64°C, and PCR products were visualised on a 1% (w/v) agarose gel. The clearest bands were used as indicators for optimal annealing temperatures (**Figure 5.4**). Products with the clearest bands, and melting curves which had Ct values < 30 on average (using the previous described threshold), were distinguishable from negative controls (-RT) and no template controls (NTC), had their relative expression quantified (**Figures 5.2B** and **5.2C**). Where negative controls had relatively high concentration (Ct values < 30), Ct values for the cDNA were considered acceptable only if they were at least three Ct values below Ct values of the negative controls, to ensure background gDNA contamination did not affect the quantification.

Where two or more peaks were present or if peaks were abnormally shaped (**Figure 5.4C**), PCR products were visualised on a gel to determine if the products were primer dimers or non-specific products. Where multiple products existed, products were sequenced to determine if these were products of homoeologous chromosomes. Each of these bands were confirmed by sequencing to be from homoeologous chromosomes, and the Ct values calculated by RT-qPCR were for these multiple products collectively (the Ct total). An additional step (before florescence was measured), where a

final temperature ranged from 80 to 85°C, was added to eliminate primer dimers and non-specific products during quantification where necessary. Samples were handled in an enclosed desk space that was regularly cleaned with 10% bleach, 70% ethanol and exposed to UV light after use to minimise contamination.

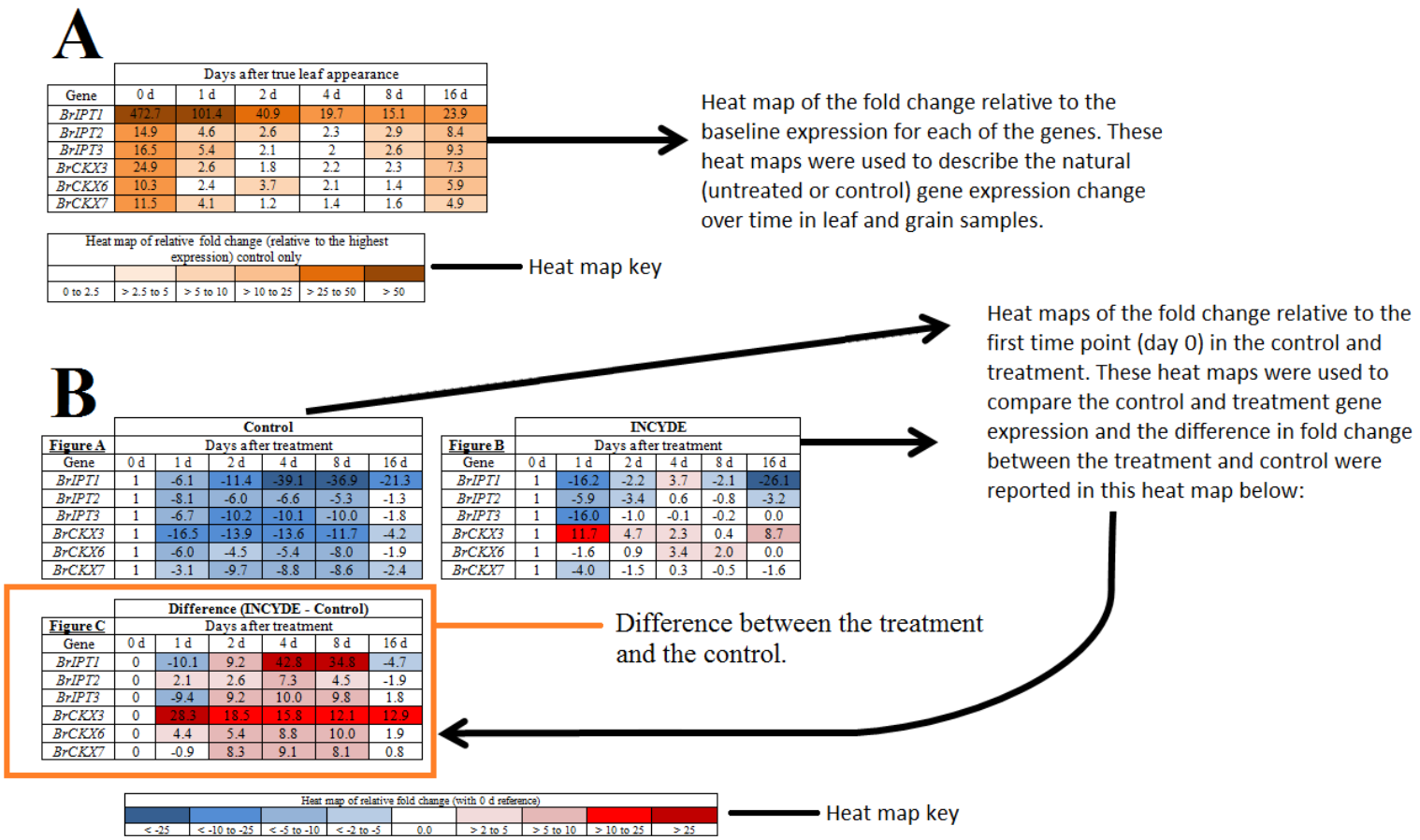

Figure 5.3 The presentation of heat maps in the results section. A The heat map generated from calculating the fold change relative to the baseline expression using control plants only. This heat map was only used for describing gene expression changes over time within each tissue. B The heat maps generated from calculating the fold change relative to the first time point for the control and treatment separately. These heat maps were used to make comparisons of the difference in fold change between the treatment and control and a direct comparison of the difference (treatment - control) was provided in each case (highlighted with an orange box). The differences between the heat maps will be discussed.

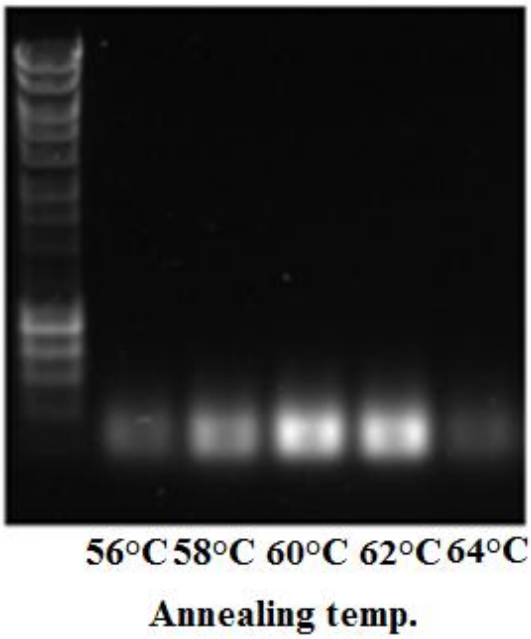

Figure 5.4 Annealing temperature optimisation by gel inspection with temperatures ranging from 56 to 64°C.

5.2.11 Sequencing the PCR product

PCR products were prepared for sequencing with an UltraClean® 15 DNA Purification Kit (MO BIO Laboratories). Agarose gels containing the PCR products were cut under UV light and placed into Eppendorf tubes. Three volumes of ULTRA SALT (MO BO Laboratories) were added to each tube and the samples were mixed. Gels were melted at 55 to 65°C for 10 min. ULTRA BIND (MO BO laboratories) was resuspended by vortexing, then 6 µL was added to the tube and the sample incubated for 5 min on the bench and mixed by flicking. The sample was centrifuged (7 000 *g*, 5 s), and the supernatant removed. The pellet was resuspended in 1 mL of ULTRA WASH (MO BO laboratories) by vortexing for 5 to 10 s. Samples were centrifuged (7 000 *g*, 5 s), the supernatant was discarded and the sample centrifuged again (10 000 *g*, 1 min). All traces of ULTRA WASH were removed and samples were air dried for 10 min.

The pellet was resuspended in 12 µL of 1x Tris-EDTA buffer (Thermo Fisher Scientific) and incubated for up to 5 min at room temperature. The sample was centrifuged (10 000 *g*, 1 min), the supernatant removed and transferred to a new tube. The concentration of the product was determined with the Nanodrop™ spectrophotometer (Thermo Fisher Scientific), and confirmed on a 1% (w/v) agarose gel. If the product was > 50 ng/µL and clear on the gel, it was sent to Macrogen Inc. for sequencing. The sequences from Macrogen were then searched on Nucleotide BLAST (Basic Local Alignment Search Tool: https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the product confirmed by alignment with the target gene.

5.3 Results

5.3.1 Phylogeny of cell wall invertases

A phylogenetic tree was created to view the relationship between cell wall invertases from closely-related species, and this knowledge was used in primer design of *CWINV*s. A phylogenetic tree produced by the maximum likelihood (ML) method for cell wall invertases indicated the close relationship between *TaCWINV* genes and orthologues in closely-related species rice, maize, barley and purple false brome (**Figure 5.5**). The phylogenetic tree showed that *TaCWINV2SM* homoeologues are closely-related to *CWINV1* genes in barley and brome, while *TaCWINV1SM* is on a separate clade and more closely-related to *CWINV2* in brome, maize and rice. Identified sequences were used to successfully design primers *TaCWINV1* and *2*, and the sequences were confirmed (Appendices 5.4.1 and 5.4.2).

Figure 5.5 A phylogenetic tree of cell wall invertases. Cell wall invertases were identified in *Triticum aestivum*, rice (*Oryza sativa*), maize (*Zea mays*), barley (*Hordeum vulgare*) and purple false brome (*Brachypodium distachyon*). Trees were produced using the maximum likelihood (ML) method in MEGA7. The node values are the percentage of bootstraps generated with 1000 bootstrap replicates. The scale indicates genetic change (substitutions/site). Gene family members that were used in Primer Premier 6.23 (PREMIER Biosoft) for primer design are circled and were aligned with each primer pair in **Appendices 5.4.1** and **5.4.2**.

5.3.2 PCR products

Products from RT-qPCR analyses were visualised on 1% agarose gels for wheat and RCBr to check for the size and presence of multiple bands. Each band was at the expected size between 180 to 300 bp. Two bands were identified for each of *TaIPT2*, *7*, *TaCKX1*, *4*, *8*, *11*, *TaGLU1a*, *TaZOG2* and *TaCWINV2*. These bands were confirmed to be PCR products by sequencing and ascertained to not be non-specific products. The presence of multiple bands is summarised for wheat and RCBr in Appendix 5.3 and highlighted for genes in the gene expression heat maps below (**Figures 5.6** to **5.10**). The PCR product alignments of *TaCWINV1* and *2* are shown in Appendices 5.4.3 and 5.4.4.

5.3.3 LC-MS/MS analyses

LC-MS/MS analyses were carried out to determine the concentration of cytokinin metabolites in wheat and barley grain following PGR treatment. No analysis was carried out with wheat grain following INCYDE treatment as the wheat field trial for these samples was infected with *Septoria*. LC-MS/MS analyses show that the quantity of *t*Z at 4 daa/t is much greater than the concentration of other free bases *c*Z, iP and DHZ in both wheat and barley grains (**Table 5.3** and **5.4**). In wheat grain following TDZ-K and CPPU treatment (**Table 5.3**), ANOVA revealed that for any of the cytokinin types measured, there were no statistically significant differences between the control and either treatment (Appendix 5.1).

In developing barley grains following INCYDE treatment (**Table 5.4**), when compared to the control, there are statistically significant decreases in the content of *c*ZR ($F_{1, 2}$ = 26.23, p = 0.036, *post hoc* Dunnett test, two sided, confidence interval: 95%, Appendix 5.2.13), while following INCYDE treatment there is a significant increase in the content of *c*ZOG ($F_{1, 2}$ = 956.39, p = 0.001, *post hoc* Dunnett test, two sided, confidence interval: 95%, Appendix 5.2.14) and this corresponded in a significant increase in the total *c*Z types overall ($F_{1, 2}$ = 55.85, p = 0.017, *post hoc* Dunnett test, two sided, confidence interval: 95%, Appendix 5.2.17) and a decrease in the total free B+R *c*Z types ($F_{1, 2}$ = 28.4, p = 0.033, *post hoc* Dunnett test, two sided, confidence interval: 95%, Appendix 5.2.27). Ultimately, following INCYDE treatment, there was a statistically significant increase in the total content of cytokinin *O*-glucosides ($F_{1, 2}$ = 50.15, p = 0.019, *post hoc* Dunnett test, two sided, confidence interval: 95%, Appendix 5.2.33).

With the DHZ type cytokinins, following INCYDE treatment, there was statistically significant decrease in the content of DHZ ($F_{1, 2}$ = 43.18, p = 0.022, *post hoc* Dunnett test, two sided, confidence

interval: 95%, Appendix 5.2.18), DHZR ($F_{1, 2}$ = 20.22, p = 0.046, *post hoc* Dunnett test, two sided, confidence interval: 95%, Appendix 5.2.19), the total B+R DHZ types ($F_{1, 2}$ = 21.82, p = 0.043, *post hoc* Dunnett test, two sided, confidence interval: 95%, Appendix 5.2.28) and DHZOG ($F_{1, 2}$ = 27.43, p = 0.035, *post hoc* Dunnett test, two sided, confidence interval: 95%, Appendix 5.2.20). Cytokinins that were below the limit of detection (LOD) included *t*Z7G, iP7G, iP9G, *c*Z9G and DHZRMP.

Table 5.3 LC-MS/MS analyses of the quantity of cytokinins in wheat grains four days after anthesis (GS 60) and treatment (daa/t).

	Wheat Cytokinin concentrations (pmol/g DW)			ANOVA		
Type	Control	TDZ-K 50 µM	CPPU 100 µM	*F*-value ($F_{2, 4}$)[a]	*p*-value	Statistics
*t*Z	794.5 ± 71.1	705.3 ± 75.7	889.2 ± 73.7	1.23	0.384	**Appendix 5.1.1**
*t*ZR	60.0 ± 6.7	60.3 ± 3.2	63.5 ± 2.3	0.19	0.831	**Appendix 5.1.2**
*t*ZOG	20.4 ± 2.8	24.7 ± 0.5	22.8 ± 1.3	1.64	0.301	**Appendix 5.1.3**
*t*ZROG	5.8 ± 0.7	6.8 ± 0.2	6.5 ± 0.5	1.22	0.385	**Appendix 5.1.4**
*t*ZRMP	115.9 ± 3.6	106.5 ± 2.8	103.1 ± 10.5	2.39	0.208	**Appendix 5.1.5**
*t*Z7G	< LOD	< LOD	< LOD			
*t*Z9G	247.3 ± 21.3	286.1 ± 3.5	268.7 ± 11.5	4.27	0.102	**Appendix 5.1.6**
Total *t*Z types	1244.0 ± 104.8	1189.7 ± 85.2	1353.8 ± 57.0	0.97	0.454	**Appendix 5.1.7**
iP	1.6 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	2.65	0.185	**Appendix 5.1.8**
iPR	2.2 ± 0.2	1.8 ± 0.1	2.5 ± 0.4	2.28	0.218	**Appendix 5.1.9**
iPRMP	22.9 ± 1.5	22.1 ± 3.8	25.9 ± 4.0	0.26	0.786	**Appendix 5.1.10**
iP7G	< LOD	< LOD	< LOD			
iP9G	< LOD	< LOD	< LOD			
Total iP types	26.8 ± 1.7	25.1 ± 3.9	29.7 ± 4.1	0.32	0.741	**Appendix 5.1.11**
*c*Z	9.6 ± 0.5	7.8 ± 1.1	8.6 ± 1.2	0.78	0.519	**Appendix 5.1.12**
*c*ZR	33.9 ± 3.9	24.7 ± 3.5	30.5 ± 4.0	1.27	0.374	**Appendix 5.1.13**
*c*ZOG	114.3 ± 12.4	130.8 ± 11.7	116.1 ± 12.9	0.42	0.684	**Appendix 5.1.14**
*c*ZROG	139.1 ± 13.2	154.5 ± 6.6	150.6 ± 8.9	0.6	0.594	**Appendix 5.1.15**
*c*ZRMP	10.0 ± 0.8	7.7 ± 1.3	10.8 ± 1.2	3.33	0.141	**Appendix 5.1.16**
*c*Z9G	< LOD	< LOD	< LOD			
Total *c*Z types	306.5 ± 26.5	325.5 ± 12.2	316.5 ± 17.0	0.21	0.821	**Appendix 5.1.17**
DHZ	0.23 ± 0.01	0.20 ± 0.03	0.20 ± 0.03	0.44	0.67	**Appendix 5.1.18**
DHZR	2.9 ± 0.1	2.4 ± 0.3	2.6 ± 0.3	0.78	0.519	**Appendix 5.1.19**
DHZOG	1.4 ± 0.2	1.7 ± 0.1	1.5 ± 0.1	1.28	0.372	**Appendix 5.1.20**
DHZROG	9.5 ± 1.1	10.7 ± 0.6	10.0 ± 0.8	0.56	0.611	**Appendix 5.1.21**
DHZRMP	< LOD	< LOD	< LOD			
DHZ7G	15.0 ± 0.3	13.3 ± 0.9	14.6 ± 2.2	0.47	0.654	**Appendix 5.1.22**
DHZ9G	0.07 ± 0.003	0.06 ± 0.01	0.07 ± 0.01	0.25	0.79	**Appendix 5.1.23**
Total DHZ types	29.1 ± 1.5	28.2 ± 0.7	28.9 ± 2.6	0.06	0.94	**Appendix 5.1.24**
B+R *t*Z types	854.5 ± 77.7	765.6 ± 78.9	952.6 ± 75.9	1.16	0.401	**Appendix 5.1.25**
B+R iP types	3.9 ± 0.3	3.1 ± 0.1	3.8 ± 0.5	2.25	0.221	**Appendix 5.1.26**
B+R *c*Z types	42.1 ± 5.3	32.5 ± 4.7	39.1 ± 5.1	0.69	0.553	**Appendix 5.1.27**
B+R DHZ types	3.1 ± 0.1	2.6 ± 0.3	2.8 ± 0.3	0.73	0.538	**Appendix 5.1.28**
Total active CKs (B+R)	904.7 ± 78.1	803.7 ± 83.9	998.3 ± 81.8	1.13	0.408	**Appendix 5.1.29**
Total CK bases	806.0 ± 71.4	714.6 ± 76.9	899.3 ± 74.9	1.21	0.388	**Appendix 5.1.30**
Total CK ribosides	98.7 ± 6.8	89.1 ± 7.0	99.0 ± 7.0	0.56	0.612	**Appendix 5.1.31**
Total CK nucleotides	148.9 ± 3.7	136.3 ± 2.0	139.8 ± 12.8	0.92	0.468	**Appendix 5.1.32**
Total CK *O*-glucosides	290.5 ± 30.2	329.2 ± 18.8	307.5 ± 23.7	0.53	0.623	**Appendix 5.1.33**
Total CK *N*-glucosides	262.3 ± 21.6	299.4 ± 3.9	283.4 ± 13.6	3.81	0.119	**Appendix 5.1.34**
Total cytokinins	1606.4 ± 117.3	1568.5 ± 69.5	1728.9 ± 43.4	1.11	0.415	**Appendix 5.1.35**

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

Grain samples were taken from the wheat cultivar Torch field trial (2014/15), and analysed as described in section **5.2.2**. Statistical analyses were carried out with ANOVA and the *F*-values and *p*-values reported. The ANOVA is described in the Appendices. The overall means for treatment levels were generated from three independent replicates (n = 3), with samples acquired from separate blocks, arranged in a randomised complete block trial. LOD indicates below limit of detection. The full name of each cytokinin is provided in the Abbreviations section. Since these were acquired from the field trials, no samples were acquired for INCYDE as the Orator field trial (which was targeted with INCYDE) was infected with *Septoria*.

Table 5.4 LC-MS/MS analyses of the quantity of cytokinins in barley grains four days after anthesis (GS 60) and treatment (daa/t).

	Barley Cytokinin concentrations (pmol/g DW)		ANOVA		
Type	Control	INCYDE 50 µM	*F*-value ($F_{1, 2}$)[a]	*p*-value	Statistics
*t*Z	759.5 ± 66.8	642.8 ± 30.0	2.58	0.249	**Appendix 5.2.1**
*t*ZR	432.0 ± 44.3	458.9 ± 36.6	0.11	0.771	**Appendix 5.2.2**
*t*ZOG	89.5 ± 2.7	97.6 ± 7.9	2.32	0.267	**Appendix 5.2.3**
*t*ZROG	28.1 ± 1.8	30.2 ± 0.6	2.5	0.255	**Appendix 5.2.4**
*t*ZRMP	455.3 ± 27.0	454.6 ± 13.0	< 0.001	0.986	**Appendix 5.2.5**
*t*Z7G	< LOD	< LOD			
*t*Z9G	46.2 ± 2.6	65.0 ± 9.1	2.82	0.235	**Appendix 5.2.6**
Total *t*Z types	1810.6 ± 142.1	1749.1 ± 61.6	0.1	0.781	**Appendix 5.2.7**
iP	2.0 ± 0.2	2.3 ± 0.3	0.78	0.47	**Appendix 5.2.8**
iPR	4.5 ± 0.6	4.7 ± 0.3	0.18	0.71	**Appendix 5.2.9**
iPRMP	86.8 ± 12.8	89.0 ± 11.9	0.11	0.776	**Appendix 5.2.10**
iP7G	< LOD	< LOD			
iP9G	< LOD	< LOD			
Total iP types	93.3 ± 13.6	96.0 ± 12.2	0.14	0.746	**Appendix 5.2.11**
*c*Z	3.6 ± 0.1	3.2 ± 0.1	4.02	0.183	**Appendix 5.2.12**
*c*ZR	23.4 ± 0.5	**20.1 ± 0.5***	26.23	**0.036**	**Appendix 5.2.13**, Levene's *p*-value: 0.918
*c*ZOG	328.9 ± 11.8	**417.1 ± 9.7***	956.39	**0.001**	**Appendix 5.2.14**, Levene's *p*-value: 0.849
*c*ZROG	242.5 ± 8.4	256.8 ± 8.6	1.34	0.367	**Appendix 5.2.15**
*c*ZRMP	20.0 ± 1.8	20.6 ± 1.9	0.04	0.858	**Appendix 5.2.16**
*c*Z9G	< LOD	< LOD			
Total *c*Z types	618.4 ± 18.8	**717.9 ± 12.8***	55.85	**0.017**	**Appendix 5.2.17**, Levene's *p*-value: 0.669
DHZ	0.9 ± 0.02	**0.7 ± 0.04***	43.18	**0.022**	**Appendix 5.2.18**, Levene's *p*-value: 0.497
DHZR	6.5 ± 0.1	**5.1 ± 0.3***	20.22	**0.046**	**Appendix 5.2.19**, Levene's *p*-value: 0.253
DHZOG	12.6 ± 0.6	**10.6 ± 0.4***	27.43	**0.035**	**Appendix 5.2.20**, Levene's *p*-value: 0.668
DHZROG	47.3 ± 3.1	43.0 ± 2.8	12.22	0.073	**Appendix 5.2.21**
DHZRMP	< LOD	< LOD			
DHZ7G	6.0 ± 0.3	5.0 ± 0.7	4.35	0.172	**Appendix 5.2.22**
DHZ9G	0.06 ± 0.01	0.06 ± 0.01	0.11	0.770	**Appendix 5.2.23**
Total DHZ types	73.0 ± 3.7	64.5 ± 3.8	12.06	0.074	**Appendix 5.2.24**
B+R *t*Z types	1191.5 ± 109.5	1101.7 ± 53.0	0.34	0.617	**Appendix 5.2.25**
B+R iP types	6.4 ± 0.8	7.0 ± 0.6	0.37	0.605	**Appendix 5.2.26**
B+R *c*Z types	27.0 ± 0.4	**23.3 ± 0.6***	28.4	**0.033**	**Appendix 5.2.27**, Levene's *p*-value: 0.834
B+R DHZ types	7.4 ± 0.1	**5.8 ± 0.3***	21.82	**0.043**	**Appendix 5.2.28**, Levene's *p*-value: 0.232
Total active CKs (B+R)	1232.2 ± 110.0	1137.8 ± 54.3	0.37	0.604	**Appendix 5.2.29**
Total CK bases	766.0 ± 67.0	649.0 ± 30.4	2.55	0.251	**Appendix 5.2.30**
Total CK ribosides	466.3 ± 44.6	488.8 ± 37.1	0.08	0.809	**Appendix 5.2.31**
Total CK nucleotides	562.2 ± 38.6	564.2 ± 11.1	< 0.001	0.966	**Appendix 5.2.32**
Total CK *O*-glucosides	748.9 ± 27.8	**855.4 ± 25.2***	50.15	**0.019**	**Appendix 5.2.33**, Levene's *p*-value: 0.886
Total CK *N*-glucosides	51.9 ± 2.6	70.1 ± 8.7	2.79	0.237	**Appendix 5.2.34**
Total cytokinins	2595.2 ± 177.1	2627.5 ± 68.9	0.02	0.894	**Appendix 5.2.35**

[a] *F*-values are provided with degrees of freedom (df) of each factor and the within group (error) in the format (*F* df factor, df within error).

* Indicates a statistically significant difference (Dunnett, two sided, confidence interval: 95%) for a treatment compared to the control.

Grain samples were taken from the barley cultivar Quench field trial (2014/15), and analysed as described in section **5.2.2**. Statistical analyses were carried out with ANOVA and the *F*-values and *p*-values reported. Where the *p*-value for the treatment factor was ≤ 0.05, a *post hoc* Dunnett test was carried out and statistically significant differences between the control and treatment reported. The ANOVA and *post hoc* Dunnett tests are described in the Appendices. The overall means for treatment levels were generated from three independent replicates (n = 3), with samples acquired from separate blocks, arranged in a randomised complete block trial. LOD indicates below limit of detection. The full name of each cytokinin is provided in the Abbreviations section. Only a single field trial for barley was carried out, and only INCYDE was applied onto this trial in Chapter 3.

5.3.4 Gene expression in wheat following anthesis

When describing and discussing natural expression pattern trends of leaf and grain samples, only heat maps that compare expression levels with the baseline expression, that is **Figure 5.6** for wheat and **Figures 5.11** and **5.15** for RCBr, will be used to describe and discuss natural changes in expression over time in untreated (control) plants.

Heat maps of the relative expression of genes in control wheat plants (**Figure 5.6**), showed the strongest elevation in expression, relative to the baseline expression, for members of *TaIPT*, *TaCKX* gene families and genes *TaRRA4*, *TaZOG2* and *TaCWINV2* in developing grain at 0 to 1 days after anthesis (daa). Elevated expression at 0 to 1 daa was particularly evident for *TaIPT3*, *5* and *7*. Expression levels dropped for most *TaIPT*s and *TaCKX*s (with the exception of *TaCKX1*) around 2 to 4 daa before showing a small increase again after 4 daa. The expression of *TaCKX1*, *TaGLU1a* and *TaCWINV1* showed different expression trends, with each member reaching a peak in expression between 4 to 7 daa in grain before dropping in expression by 14 daa. Strong increases in expression in both grain and flag leaf samples were attained in *TaGLU1a* between 0 and 7 daa.

In flag leaf samples, little expression change occurred with the *TaIPT*s (**Figure 5.6**), with the exception of *TaIPT7*, which peaked in expression between 2 to 4 daa. The expression of *TaCKX*s was in comparison, generally more sustained, with *TaCKX1* and *2* peaking in expression at 7 daa, and *TaCKX8*, *10* and *11* expressing strongest, compared to the baseline expression, between 1 to 4 daa. Expression of *TaZOG2* was relatively sustained (except at 1 daa) over the week measured, and expressed much stronger in flag leaves compared to grain.

	Days after anthesis (daa)										
	Wheat leaf					Wheat grain					
Gene	0 d	1 d	2 d	4 d	7 d	0 d	1 d	2 d	4 D	7 d	14 d
*TaIPT2**	1.3	3	2.4	2.6	2.2	3	1.5	1.3	5.6	2.8	6.7
TaIPT3	1.4	2.5	1.8	2.2	5.4	4.8	40	2.7	1.9	2	4.2
TaIPT5	2.4	2.4	2.9	1.3	1.4	28.2	41.1	23.7	3	7.7	6.2
*TaIPT7**	2.7	6.2	27.8	20.3	10.1	10.7	5	2.8	2.3	3.3	5.8
TaIPT8	1.3	2.4	2.2	1.1	1.9	3.7	5	2.9	2	1.7	2.2
*TaCKX1**	2	3.5	2.6	3.7	7.1	2	5.8	6.9	7.5	11.5	2.5
TaCKX2	2.1	2.1	2.7	2.2	15.6	3.1	2.7	1.2	2.2	2.7	1.4
TaCKX3	1.2	3.6	2.6	2.6	2.2	5.7	7.7	1.8	1.6	3.9	10.8
*TaCKX4**	1.8	1.8	2.1	1.5	1.9	3.4	4.3	1.2	2.2	3.1	7.2
*TaCKX8**	2.1	10.1	9.7	4.7	1.8	1.9	4.7	1.6	5	3.7	3.7
TaCKX10	1.8	7.5	4.6	3.8	1.9	2.9	2.8	2	5.9	3.8	7.1
*TaCKX11**	1.2	3	6.8	2.5	1.9	1.4	2.6	2.1	4.2	2.8	5.9
*TaGLU1a**	3.7	35.7	2	31.6	4.8	8.4	9.6	51.7	61	27.2	1.8
TaRRA4	1.3	1.6	2	1.7	2.8	7.5	5.2	1.7	2.5	2.5	2.9
*TaZOG2**	11.4	1.7	11.1	12.1	6.3	5	2.7	2.1	1.9	1.5	3.4
TaCWINV1						1.9	9.1	3.7	6.3	11.9	1.6
*TaCWINV2**	1.9	1.4	1.2	1.5	2.1	5.3	5.4	2.5	1.7	2.1	3.4

Heat map of relative fold change (relative to baseline expression) in control only					
0 to 2.5	> 2.5 to 5	> 5 to 10	> 10 to 25	> 25 to 50	> 50

Figure 5.6 Heat map of the relative expression of genes in wheat grain and leaf samples relative to the baseline expression in control plants. Samples were taken at 0 to 14 d after anthesis with grain from wheat cv. Torch and 0 to 7 d after anthesis with flag leaves from with wheat cv. Morph after anthesis (daa) (GS 61). The relative expression is represented by a heat map in the legend. * Indicates the presence of multiple PCR products for the gene (see **Appendix 5.3**). See section **5.2.9** for more details.

5.3.5 Gene expression in wheat after PGR treatment

For comparisons between the control and PGR-treated plants, heat maps where day 0 is taken as a reference for expression level will be used, that is **Figures 5.7** and **5.8** for wheat flag leaf, **Figures 5.9** and **5.10** for wheat grain and **Figures 5.12**, **5.13** and **5.14** for RCBr. For these comparison heat maps, whether gene family members are upregulated or downregulated (relative to 0 d) will be compared between the control and treatment. See section **5.2.9** for more details on how these heat maps were constructed, or **Figure 5.3** for how these heat maps are presented.

5.3.5.1 Wheat flag leaves

The flag leaves were taken from pot trials where INCYDE and TDZ-K were targeted onto wheat plants (see section **5.2.1.2**). In the wheat flag leaves, following INCYDE treatment, *TaCKX8* and *TaGLU1a* upregulation (based on the 0 d expression level) was sustained and stable (**Figure 5.7B**) for longer in comparison to the control (**Figures 5.7A** and **5.7C**). In contrast, there was a downregulation of *TaIPT2* following INCYDE treatment (**Figure 5.7B**) in comparison to the upregulation for the control (**Figures 5.7A** and **5.7C**). While there were similar levels of upregulation of most *TaIPT*s and *TaCKX*s at 2 days after treatment between the control (**Figure 5.7A**) and treatment (**Figure 5.7B**), at 4 days after INCYDE treatment there was a low levels of upregulation and downregulation (based on the 0 d expression level), compared to the control; where there was an upregulation of most *TaIPT*s and *TaCKX*s (**Figures 5.7A** and **5.7C**). The expression of *TaRRA4* was upregulated more strongly following INCYDE treatment (**Figure 5.7B**) in comparison to the control at 1, 2 and 7 days after treatment (**Figures 5.7A** and **5.7C**).

Treating wheat plants with TDZ-K had the effect of downregulating (based on the 0 d expression level) and reducing the upregulation of *TaIPT2*, *3* and *5* in flag leaves after treatment (**Figure 5.8B**), in comparison to the control where these gene family members showed upregulation or less downregulation (**Figures 5.8A** and **5.8C**). Treatment with TDZ-K also resulted in reduced upregulation at 1 day after treatment of *TaCKX8*, *10* and *11* (**Figure 5.8B**), in comparison to the stronger upregulation in the control (**Figures 5.8A** and **5.8C**). TDZ-K treatment also had the effect of increasing the upregulation of *TaCKX1* and *2* at 1 and 2 days after treatment (**Figure 5.8B**) in comparison to the upregulation in the control (**Figures 5.8A** and **5.8C**). The expression of *TaGLU1a* was downregulated immediately following TDZ-K treatment (**Figure 5.8B**) in comparison to the control where it was strongly upregulated (**Figures 5.8A** and **5.8C**). There was a downregulation of *TaRRA4* following TDZ-K treatment at 2 days after treatment (**Figure 5.8B**), compared to the

upregulation in the control leaves (**Figures 5.8A** and **5.8C**). While with *TaZOG2*, at 7 days after treatment, there was a reduced downregulation of expression following TDZ-K treatment (**Figure 5.8B**) compared to the control (**Figures 5.8A** and **5.8C**).

Figure A

	Control				
	Days after anthesis and treatment (daa/t)				
Gene	0 d	1 d	2 d	4 d	7 d
*TaIPT2**	1	2	1.7	2.2	1.1
TaIPT3	1	2	1.0	0.3	2.5
TaIPT5	1	-0.3	1.7	-1.9	-1.9
*TaIPT7**	1	2.5	3.1	1.3	0.3
TaIPT8	1	1.9	1.8	-0.4	1.1
*TaCKX1**	1	1.9	1.3	1.3	2.5
TaCKX2	1	0.9	1.4	1.9	3.7
TaCKX3	1	3.1	2.3	2.3	1.9
*TaCKX4**	1	0.4	0.6	-0.3	0.1
*TaCKX8**	1	4.2	6.8	2.4	-0.6
TaCKX10	1	4.2	2.7	2.6	0.1
*TaCKX11**	1	2.8	3.9	1.6	1.6
*TaGLU1a**	1	7.6	-2.2	8	0.8
TaRRA4	1	0.1	1.2	0.3	2.4
*TaZOG2**	1	-1.7	-0.2	0.0	-6.1
*TaCWINV2**	1	-1.5	-1.6	-0.7	0.1

Figure B

	INCYDE				
	Days after anthesis and treatment (daa/t)				
Gene	0 d	1 d	2 d	4 d	7 d
*TaIPT2**	1	0.3	-1.4	0.8	-1.7
TaIPT3	1	2.1	1.1	-0.4	0.0
TaIPT5	1	3.1	2	-0.1	0.7
*TaIPT7**	1	4.1	3	-0.2	2
TaIPT8	1	2	2	0.3	1.6
*TaCKX1**	1	0.1	2	-0.9	2.4
TaCKX2	1	0.4	-0.2	-1.8	0.3
TaCKX3	1	1.4	1.6	2.6	-1.7
*TaCKX4**	1	0.0	0.7	-4	1.3
*TaCKX8**	1	3.1	5.9	7.1	7.4
TaCKX10	1	1.4	6.1	0.0	2.4
*TaCKX11**	1	2.7	4.6	0.0	1.7
*TaGLU1a**	1	3.1	4.5	4.2	6.6
TaRRA4	1	1.6	2.5	0.0	5.2
*TaZOG2**	1	0.6	0.6	-0.4	-1.2
*TaCWINV2**	1	1.8	0.6	1.9	0.0

Figure C

	Difference (INCYDE - Control)				
	Days after anthesis and treatment (daa/t)				
Gene	0 d	1 d	2 d	4 d	7 d
*TaIPT2**	0	-1.6	-3.2	-1.3	-2.8
TaIPT3	0	0.1	0.1	-0.7	-2.5
TaIPT5	0	3.4	0.3	1.8	2.6
*TaIPT7**	0	1.6	-0.1	-1.4	1.7
TaIPT8	0	0.1	0.2	0.8	0.6
*TaCKX1**	0	-1.8	0.7	-2.2	-0.2
TaCKX2	0	-0.5	-1.5	-3.7	-3.4
TaCKX3	0	-1.7	-0.7	0.3	-3.5
*TaCKX4**	0	-0.4	0.1	-3.8	1.2
*TaCKX8**	0	-1.1	-1.0	4.7	8.0
TaCKX10	0	-2.9	3.4	-2.7	2.3
*TaCKX11**	0	-0.1	0.6	-1.6	0.1
*TaGLU1a**	0	-4.5	6.7	-3.9	5.8
TaRRA4	0	1.5	1.4	-0.3	2.9
*TaZOG2**	0	2.4	0.8	-0.4	5.0
*TaCWINV2**	0	3.3	2.2	2.6	-0.1

Figure 5.7 Heat map of the relative expression in wheat leaves compared to 0 d in the control and INCYDE-treated plants. A: Heat map of control plants, B: heat map of plants following 50 µM INCYDE treatment, C: the difference in expression between the INCYDE treatment (B) and the control (A), with treatment minus the control presented. Flag leaf samples were taken at 0 to 7 d after anthesis (daa) (GS 61) and treatment (daa/t). The relative expression is represented by a heat map in the legend. * Indicates the presence of multiple PCR products for the gene (see **Appendix 5.3**). See section **5.2.9** for more details.

Heat map of relative fold change in respect to 0 d								
< -8	< -4 to -8	< -2 to -4	< -1 to -2	0.0	> 1 to 2	> 2 to 4	> 4 to 8	> 8

Figure A

	Control				
	Days after anthesis and treatment (daa/t)				
Gene	0 d	1 d	2 d	4 d	7 d
*TaIPT2**	1	2	1.7	2.2	1.1
TaIPT3	1	2	1.0	0.3	2.5
TaIPT5	1	-0.3	1.7	-1.9	-1.9
*TaIPT7**	1	2.5	3.1	1.3	0.3
TaIPT8	1	1.9	1.8	-0.4	1.1
*TaCKX1**	1	1.9	1.3	1.3	2.5
TaCKX2	1	0.9	1.4	1.9	3.7
TaCKX3	1	3.1	2.3	2.3	1.9
*TaCKX4**	1	0.4	0.6	-0.3	0.1
*TaCKX8**	1	4.2	6.8	2.4	-0.6
TaCKX10	1	4.2	2.7	2.6	0.1
*TaCKX11**	1	2.8	3.9	1.6	1.6
*TaGLU1a**	1	7.6	-2.2	8	0.8
TaRRA4	1	0.1	1.2	0.3	2.4
*TaZOG2**	1	-1.7	-0.2	0.0	-6.1
*TaCWINV2**	1	-1.5	-1.6	-0.7	0.1

Figure B

	TDZ-K				
	Days after anthesis and treatment (daa/t)				
Gene	0 d	1 d	2 d	4 d	7 d
*TaIPT2**	1	-1.8	-1.1	-2.3	-2.5
TaIPT3	1	0.7	-0.9	-0.8	-1.5
TaIPT5	1	-2	-1.6	1.6	-2.2
*TaIPT7**	1	4.4	4.3	3.5	1.0
TaIPT8	1	-0.4	1.6	0.1	-0.4
*TaCKX1**	1	3	2.6	1.8	3
TaCKX2	1	2.3	6.7	0.2	1.2
TaCKX3	1	2	3.1	5	-0.5
*TaCKX4**	1	1.1	-0.4	-2.7	0.0
*TaCKX8**	1	0.4	0.2	1.5	1.6
TaCKX10	1	1.7	2.8	-1.4	-1.9
*TaCKX11**	1	2.2	2.6	3.3	0.2
*TaGLU1a**	1	-0.5	-1.6	1.8	2.2
TaRRA4	1	-0.4	-1.4	-5.7	-1.2
*TaZOG2**	1	-5.3	-1.2	1.1	-1.5
*TaCWINV2**	1	-3	-2.3	-0.5	-2.4

Figure C

	Difference (TDZ-K - Control)				
	Days after anthesis and treatment (daa/t)				
Gene	0 d	1 d	2 d	4 d	7 d
*TaIPT2**	0	-3.8	-2.9	-4.4	-3.6
TaIPT3	0	-1.3	-1.9	-1.1	-4.1
TaIPT5	0	-1.6	-3.2	3.5	-0.3
*TaIPT7**	0	1.9	1.2	2.2	0.7
TaIPT8	0	-2.3	-0.3	0.5	-1.5
*TaCKX1**	0	1.1	1.3	0.5	0.4
TaCKX2	0	1.4	5.4	-1.7	-2.5
TaCKX3	0	-1.1	0.8	2.7	-2.4
*TaCKX4**	0	0.7	-1.0	-2.4	-0.1
*TaCKX8**	0	-3.9	-6.7	-0.9	2.2
TaCKX10	0	-2.5	0.1	-4.0	-2.0
*TaCKX11**	0	-0.6	-1.4	1.6	-1.3
*TaGLU1a**	0	-8.1	0.7	-6.3	1.4
TaRRA4	0	-0.5	-2.6	-6.0	-3.5
*TaZOG2**	0	-3.6	-1.0	1.0	4.6
*TaCWINV2**	0	-1.5	-0.7	0.2	-2.5

Figure 5.8 Heat map of the relative expression in wheat leaves compared to 0 d in the control and TDZ-K-treated plants. A: Heat map of control plants, B: heat map of plants following 50 µM TDZ-K treatment, C: the difference in expression between the TDZ-K treatment (B) and the control (A), with treatment minus the control presented. Flag leaf samples were taken at 0 to 7 d after anthesis (daa) (GS 61) and treatment (daa/t). The relative expression is represented by a heat map in the legend. * Indicates the presence of multiple PCR products for the gene (see **Appendix 5.3**). See section **5.2.9** for more details.

Heat map of relative fold change in respect to 0 d								
< -8	< -4 to -8	< -2 to -4	< -1 to -2	0.0	> 1 to 2	> 2 to 4	> 4 to 8	> 8

5.3.5.2 Wheat grain

As these samples were acquired from the wheat field trials, and the trial where INCYDE was targeted was infected with *Septoria*, samples were only acquired following TDZ-K and CPPU targeted at anthesis (see section **5.2.1.1**). Following TDZ-K treatment, there was a reduction in the upregulation (based on the 0 d expression level) of *TaIPT3* at 1 day after treatment (**Figure 5.9B**) compared to the control (**Figures 5.9A** and **5.9C**). There was a upregulation of *TaIPT*s *3*, *5*, *7* and *8* from 2 days after TDZ-K treatment (**Figure 5.9B**), this contrasts with the downregulation of *TaIPT*s in the control group (**Figures 5.9A** and **5.9C**). There was a strong upregulation of *TaCKX8* at 4 days after TDZ-K treatment (**Figure 5.9B**), in comparison to the control where there was a small downregulation at this point in time (**Figures 5.9A** and **5.9C**). Following TDZ-K treatment, there was no upregulation of *TaCKX10* and *11* by 14 days (**Figure 5.9B**), in contrast to the control group where there was an upregulation of both gene family members (**Figures 5.9A** and **5.9C**). There was more upregulation and less downregulation of *TaGLU1a*, *TaRRA4*, *TaZOG2* and *TaCWINV2* following TDZ-K treatment (**Figure 5.9A**), in comparison to the control (**Figures 5.9A** and **5.9C**), while with *TaCWINV1*, TDZ-K treatment resulted in a reduced upregulation at 1 day and 7 days after treatment (**Figure 5.9B**) compared to the control (**Figures 5.9A** and **5.9C**).

In grain samples of plants treated with CPPU, there was a reduced downregulation and even upregulation (based on the 0 d expression level) of *TaIPT3*, *5* and *7* from 2 days after treatment (**Figure 5.10B**) in comparison to the control which showed strong downregulation of these gene family members (**Figures 5.9A** and **5.9C**). Conversely, there was a reduction in the upregulation of *TaIPT3* at day 1 after treatment (**Figure 5.10B**) in comparison to the strong upregulation in the control (**Figures 5.9A** and **5.9C**). Between 2 and 7 days after treatment, CPPU treatment resulted in an upregulation of *TaCKX3* and *4* (**Figure 5.10B**), whereas in the control these gene family members were downregulated (**Figures 5.9A** and **5.9C**). Between days 4 and 14 there was a downregulation and reduction in upregulation in *TaCKX10* and *11* following CPPU treatment (**Figure 5.10B**), in comparison with the control where there was an upregulation of these gene family members (**Figures 5.9A** and **5.9C**). Following CPPU treatment there was also an upregulation and reduced downregulation in the expression of *TaRRA4* and *TaCWINV2* at different points over the two week period (**Figure 5.10B**) and a reduction in the upregulation of expression, compared to the control, for *TaCWINV1* at 1 and 7 days after treatment (**Figures 5.10A** and **5.10C**).

Figure A

	Control					
	Days after anthesis and treatment (daa/t)					
Gene	0 d	1 d	2 d	4 d	7 d	14 d
*TaIPT2**	1	0.5	-2.1	2	0.2	3.4
TaIPT3	1	8.9	-3.8	-3.1	-3.1	-1.1
TaIPT5	1	0.6	-2.3	-12	-4.6	-3.3
*TaIPT7**	1	-3	-6.6	-7	-4.6	-2.4
TaIPT8	1	-0.4	0.0	-1.4	-1.8	-1.2
*TaCKX1**	1	3.7	3.9	3.6	6.1	-0.3
TaCKX2	1	-0.1	-1.6	0.0	0.8	-1.9
TaCKX3	1	1.4	-2.5	-0.9	-1.3	1.2
*TaCKX4**	1	2.1	-2.9	-1.2	0.5	3.6
*TaCKX8**	1	0.3	-0.3	-1.1	1.5	2
TaCKX10	1	-1.8	-0.6	1.7	1.0	3
*TaCKX11**	1	1.9	1.0	3.2	3.4	7.8
*TaGLU1a**	1	0.7	5.6	6	3.9	-7
TaRRA4	1	-0.1	-4.5	-3.5	-2.8	-3.1
*TaZOG2**	1	-2.1	-2.3	-2.4	-3.9	-1.3
TaCWINV1	1	6.6	2.4	3.2	8	-0.3
*TaCWINV2**	1	0.3	-3.3	-2.4	-1.1	-2.6

Figure B

	TDZ-K					
	Days after anthesis and treatment (daa/t)					
Gene	0 d	1 d	2 d	4 d	7 d	14 d
*TaIPT2**	1	0.2	-0.1	0.6	0.1	-0.8
TaIPT3	1	1.6	3	2.7	1.9	4.8
TaIPT5	1	4.2	1.6	2.8	0.8	3.3
*TaIPT7**	1	2.4	1.7	1.7	1.3	4.2
TaIPT8	1	-0.2	2.1	0.2	2.2	0.5
*TaCKX1**	1	2.2	2.8	7.5	6.1	0.9
TaCKX2	1	1.7	0.1	0.8	1.3	-1.3
TaCKX3	1	1.0	0.6	-0.2	-1.0	1.7
*TaCKX4**	1	0.5	-1.3	-2.3	-1.8	0.1
*TaCKX8**	1	-1.4	0.6	4.5	2.3	2
TaCKX10	1	-1.9	-0.3	1.2	-1.2	-2.8
*TaCKX11**	1	1.2	0.7	-0.7	0.2	0.0
*TaGLU1a**	1	0.2	7.5	8.9	7.9	-2.9
TaRRA4	1	-0.2	-2.1	-1.8	-1.1	-2.6
*TaZOG2**	1	1.3	0.5	0.4	-0.6	-0.1
TaCWINV1	1	1.0	2.8	3	3	0.4
*TaCWINV2**	1	1.2	-1.2	-1.1	1.0	0.0

Figure C

	Difference (TDZ-K - Control)					
	Days after anthesis and treatment (daa/t)					
Gene	0 d	1 d	2 d	4 d	7 d	14 d
*TaIPT2**	0	-0.3	2.1	-1.5	-0.1	-4.2
TaIPT3	0	-7.3	6.8	5.8	5.1	5.9
TaIPT5	0	3.5	3.9	14.7	5.4	6.6
*TaIPT7**	0	5.4	8.3	8.7	5.9	6.6
TaIPT8	0	0.2	2.1	1.6	4	1.7
*TaCKX1**	0	-1.5	-1.1	3.8	0.0	1.2
TaCKX2	0	1.8	1.7	0.8	0.5	0.6
TaCKX3	0	-0.4	3.1	0.7	0.3	0.6
*TaCKX4**	0	-1.6	1.6	-1.1	-2.3	-3.5
*TaCKX8**	0	-1.7	1.0	5.6	0.8	0.0
TaCKX10	0	-0.1	0.3	-0.5	-2.1	-5.9
*TaCKX11**	0	-0.6	-0.3	-3.9	-3.2	-7.8
*TaGLU1a**	0	-0.5	1.9	2.9	4	4.1
TaRRA4	0	-0.1	2.3	1.7	1.7	0.6
*TaZOG2**	0	3.4	2.8	2.8	3.3	1.2
TaCWINV1	0	-5.6	0.4	-0.2	-5	0.7
*TaCWINV2**	0	0.9	2.2	1.2	2.1	2.7

Figure 5.9 Heat map of the relative expression in grain compared to 0 d in the control and TDZ-K-treated plants. A: Heat map of control plants, B: heat map of plants following 50 µM TDZ-K treatment, C: the difference in expression between the TDZ-K treatment (B) and the control (A), with treatment minus the control presented. Grain samples were taken at 0 to 14 d after anthesis (daa) (GS 61) and treatment (daa/t). The relative expression is represented by a heat map in the legend. * Indicates the presence of multiple PCR products for the gene (see **Appendix 5.3**). See section **5.2.9** for more details.

Heat map of relative fold change in respect to 0 d								
< -8	< -4 to -8	< -2 to -4	< -1 to -2	0.0	> 1 to 2	> 2 to 4	> 4 to 8	> 8

Figure A

	Control					
	Days after anthesis and treatment (daa/t)					
Gene	0 d	1 d	2 d	4 d	7 d	14 d
*TaIPT2**	1	0.5	-2.1	2	0.2	3.4
TaIPT3	1	8.9	-3.8	-3.1	-3.1	-1.1
TaIPT5	1	0.6	-2.3	-12	-4.6	-3.3
*TaIPT7**	1	-3	-6.6	-7	-4.6	-2.4
TaIPT8	1	-0.4	0.0	-1.4	-1.8	-1.2
*TaCKX1**	1	3.7	3.9	3.6	6.1	-0.3
TaCKX2	1	-0.1	-1.6	0.0	0.8	-1.9
TaCKX3	1	1.4	-2.5	-0.9	-1.3	1.2
*TaCKX4**	1	2.1	-2.9	-1.2	0.5	3.6
*TaCKX8**	1	0.3	-0.3	-1.1	1.5	2
TaCKX10	1	-1.8	-0.6	1.7	1.0	3
*TaCKX11**	1	1.9	1.0	3.2	3.4	7.8
*TaGLU1a**	1	0.7	5.6	6	3.9	-7
TaRRA4	1	-0.1	-4.5	-3.5	-2.8	-3.1
*TaZOG2**	1	-2.1	-2.3	-2.4	-3.9	-1.3
TaCWINV1	1	6.6	2.4	3.2	8	-0.3
*TaCWINV2**	1	0.3	-3.3	-2.4	-1.1	-2.6

Figure B

	CPPU					
	Days after anthesis and treatment (daa/t)					
Gene	0 d	1 d	2 d	4 d	7 d	14 d
*TaIPT2**	1	-0.2	-1.5	-1.4	-0.5	0.4
TaIPT3	1	0.5	0.7	-0.6	1.9	1.3
TaIPT5	1	3.4	0.2	-1.4	1.6	-0.2
*TaIPT7**	1	-0.2	1.5	1.6	-0.4	3.8
TaIPT8	1	0.0	-0.4	-0.7	-0.8	-2.2
*TaCKX1**	1	0.5	4	5.1	7.6	-1.1
TaCKX2	1	-1.0	0.9	0.9	1.9	-0.8
TaCKX3	1	1.9	1.7	3.6	1.6	2.9
*TaCKX4**	1	3.4	4.2	8.9	5.4	3.5
*TaCKX8**	1	0.4	1.2	0.5	-1.8	0.5
TaCKX10	1	0.0	-1.5	-1.6	-0.5	-4.9
*TaCKX11**	1	1.0	1.7	1.7	0.2	0.9
*TaGLU1a**	1	0.6	3.1	6	4.2	-6.4
TaRRA4	1	2.4	2.3	0.5	0.2	-0.3
*TaZOG2**	1	0.3	-0.2	-3.3	-2.4	-0.9
TaCWINV1	1	1.9	2	2.2	2	-1.4
*TaCWINV2**	1	1.0	-0.5	-0.6	0.0	0.9

Figure C

	Difference (CPPU - Control)					
	Days after anthesis and treatment (daa/t)					
Gene	0 d	1 d	2 d	4 d	7 d	14 d
*TaIPT2**	0	-0.6	0.6	-3.4	-0.6	-3
TaIPT3	0	-8.4	4.6	2.5	5.1	2.5
TaIPT5	0	2.8	2.5	10.5	6.2	3.1
*TaIPT7**	0	2.8	8.1	8.6	4.2	6.2
TaIPT8	0	0.4	-0.5	0.8	1.0	-1.0
*TaCKX1**	0	-3.2	0.1	1.5	1.6	-0.8
TaCKX2	0	-0.9	2.5	0.9	1.2	1.1
TaCKX3	0	0.6	4.2	4.6	2.9	1.8
*TaCKX4**	0	1.4	7.1	10	5	-0.1
*TaCKX8**	0	0.1	1.5	1.6	-3.3	-1.5
TaCKX10	0	1.8	-0.9	-3.3	-1.5	-7.9
*TaCKX11**	0	-0.9	0.8	-1.5	-3.2	-6.9
*TaGLU1a**	0	-0.1	-2.5	0.0	0.3	0.6
TaRRA4	0	2.5	6.7	4	2.9	2.8
*TaZOG2**	0	2.4	2.1	-0.9	1.4	0.4
TaCWINV1	0	-4.7	-0.5	-1.0	-6	-1.1
*TaCWINV2**	0	0.7	2.8	1.8	1.1	3.5

Figure 5.10 Heat map of the relative expression in grain compared to 0 d in the control and CPPU-treated plants. A: Heat map of control plants, B: heat map of plants following 100 µM CPPU treatment, C: the difference in expression between the CPPU treatment (B) and the control (A), with treatment minus the control presented. Grain samples were taken at 0 to 14 d after anthesis (daa) (GS 61) and treatment (daa/t). The relative expression is represented by a heat map in the legend. * Indicates the presence of multiple PCR products for the gene (see **Appendix 5.3**). See section **5.2.9** for more details.

Heat map of relative fold change in respect to 0 d								
< -8	< -4 to -8	< -2 to -4	< -1 to -2	0.0	> 1 to 2	> 2 to 4	> 4 to 8	> 8

5.3.6 Gene expression in rapid cycling *Brassica rapa* after true leaf appearance

For the RCBr expression studies, samples were acquired from the growth rooms as described in section **5.2.1.3**. For RCBr leaves of control plants, the expression of *BrIPT*s, particularly *BrIPT1*, and the *BrCKX*s was highest, compared to the least expressed samples, at day 0 after true leaf appearance (**Figure 5.11**). For both *BrIPT* and *BrCKX* gene families, the lowest expression levels were measured between 1 to 8 days after true leaf appearance, when leaves were partially expanded and as buds developed. This was followed by an increase in expression by 16 days for both gene families.

5.3.7 Gene expression in rapid cycling *Brassica rapa* after treatment

As the growth room experiments of Chapter 2 focused on INCYDE and TDZ-K application, gene expression studies here too focused on the effect of these PGRs on RCBr. In RCBr plants treated with INCYDE, at 1 day after treatment there was a stronger downregulation (based on the 0 d expression level) in the expression of *BrIPT1* and *3* (**Figure 5.12B**) compared to the more modest downregulation of the control (**Figures 5.12A** and **5.12C**). In contrast to the control, there was an upregulation of the expression of *BrCKX3* from 1 day to 16 days after treatment (**Figure 5.12B**). From 2 days after INCYDE treatment there was a reduced downregulation and upregulation in the expression of each of the *BrIPT*s and *BrCKX*s (**Figure 5.12B**) compared to the control (**Figures 5.12A** and **5.12C**), except for *BrIPT1*and *BrIPT2*, which were more downregulated (**Figure 5.12B**) compared to the control at 16 days after treatment (**Figures 5.12A** and **5.12C**).

With plants treated with TDZ-K, there was a strong upregulation (based on the 0 d expression level) of *BrCKX3* and *BrCKX6* (**Figure 5.13**) at 1 day after treatment compared to the control where there was a downregulation of expression (**Figures 5.13A** and **5.13C**). For each of the *BrIPT* and *BrCKX* gene family members, there was an upregulation (and decreased downregulation at 4 days for *BrIPT2* and *BrIPT3*) following treatment (**Figure 5.13**) in comparison to the downregulation in the control (**Figures 5.13A** and **5.13C**).

Given the importance of the nitrate concentration in the effect of INCYDE on yield with RCBr (**Table 2.3**), the effect of nitrate on the expression of cytokinin regulatory genes was investigated. After providing plants with a stronger (10 mM) concentration of KNO_3, there was a stronger downregulation (based on the 0 d expression level) of *BrIPT1*, 2, *BrCKX3* and 7 at 1 day after treatment (**Figure 5.14B**), compared to the control (**Figures 5.14A** and **5.14C**). At 2 days after

INCYDE treatment this downregulation reduces (**Figure 5.13B**), compared to the strong downregulation in the control (**Figures 5.13A** and **5.13C**). The increase in nitrate particularly has a strong effect on the expression of *BrIPT1*, where the downregulation is greatly reduced between 4 and 8 days following treatment (**Figure 5.13B**) in comparison to the control (**Figures 5.13A** and **5.13C**).

	Days after true leaf appearance					
Gene	0 d	1 d	2 d	4 d	8 d	16 d
BrIPT1	472.7	101.4	40.9	19.7	15.1	23.9
BrIPT2	14.9	1.7	2.6	2.3	2.9	8.4
BrIPT3	16.5	5.4	2.1	2	2.6	9.3
BrCKX3	24.9	1.4	1.8	2.2	2.3	7.3
BrCKX6	10.3	1.6	3.7	2.1	1.4	5.9
BrCKX7	11.5	4.1	1.2	1.4	1.6	4.9

Heat map of relative fold change (relative to the highest expression) control only					
0 to 2.5	> 2.5 to 5	> 5 to 10	> 10 to 25	> 25 to 50	> 50

Figure 5.11 Heat map of the relative expression of genes in leaves of RCBr plants relative to the baseline expression in control plants. Samples were acquired from the appearance of true leaves at different stages: from young leaves (at 0 to 2 d), to partially expanded leaves taken before flowering (at 4 to 8 d) to expanded leaves during anthesis (16 d). The relative expression is represented by a heat map in the legend. See section **5.2.9** for more details.

Figure A

	Control					
	Days after treatment					
Gene	0 d	1 d	2 d	4 d	8 d	16 d
BrIPT1	1	-6.1	-11.4	-39.1	-36.9	-21.3
BrIPT2	1	-8.1	-6.0	-6.6	-5.3	-1.3
BrIPT3	1	-6.7	-10.2	-10.1	-10.0	-1.8
BrCKX3	1	-16.5	-13.9	-13.6	-11.7	-4.2
BrCKX6	1	-6.0	-4.5	-5.4	-8.0	-1.9
BrCKX7	1	-3.1	-9.7	-8.8	-8.6	-2.4

Figure B

	INCYDE					
	Days after treatment					
Gene	0 d	1 d	2 d	4 d	8 d	16 d
BrIPT1	1	-16.2	-2.2	3.7	-2.1	-26.1
BrIPT2	1	-5.9	-3.4	0.6	-0.8	-3.2
BrIPT3	1	-16.0	-1.0	-0.1	-0.2	0.0
BrCKX3	1	11.7	4.7	2.3	0.4	8.7
BrCKX6	1	-1.6	0.9	3.4	2.0	0.0
BrCKX7	1	-4.0	-1.5	0.3	-0.5	-1.6

Figure C

	Difference (INCYDE - Control)					
	Days after treatment					
Gene	0 d	1 d	2 d	4 d	8 d	16 d
BrIPT1	0	-10.1	9.2	42.8	34.8	-4.7
BrIPT2	0	2.1	2.6	7.3	4.5	-1.9
BrIPT3	0	-9.4	9.2	10.0	9.8	1.8
BrCKX3	0	28.3	18.5	15.8	12.1	12.9
BrCKX6	0	4.4	5.4	8.8	10.0	1.9
BrCKX7	0	-0.9	8.3	9.1	8.1	0.8

Figure 5.12 Heat map of the relative expression in RCBr leaves compared to 0 d in the control and INCYDE-treated plants. A: Heat map of control plants, B: heat map of plants following 50 µM INCYDE treatment, C: the difference in expression between the INCYDE treatment (B) and the control (A), with treatment minus the control presented. 0 d was defined as the appearance of true leaves and the time point prior to treatment. Leaves were acquired at this time point, followed by an application of INCYDE 2 h after leaf acquisition, then subsequently at 2, 4, 8 and 16 days after treatment. The relative expression is represented by a heat map in the legend. Treatments were applied to the whole plants. See section **5.2.9** for more details.

Heat map of relative fold change (with 0 d reference)								
< -25	< -10 to -25	< -5 to -10	< -2 to -5	0.0	> 2 to 5	> 5 to 10	> 10 to 25	> 25

Figure A

	Control					
	Days after treatment					
Gene	0 d	1 d	2 d	4 d	8 d	16 d
BrIPT2	1	-8.1	-6.0	-6.6	-5.3	-1.3
BrIPT3	1	-6.7	-10.2	-10.1	-10.0	-1.8
BrCKX3	1	-16.5	-13.9	-13.6	-11.7	-4.2
BrCKX6	1	-6.0	-4.5	-5.4	-8.0	-1.9
BrCKX7	1	-3.1	-9.7	-8.8	-8.6	-2.4

Figure B

	TDZ-K					
	Days after treatment					
Gene	0 d	1 d	2 d	4 d	8 d	16 d
BrIPT2	1	5.4	1.4	-2.5	0.8	1.2
BrIPT3	1	1.3	0.3	-0.9	0.0	0.8
BrCKX3	1	55.1	4.0	0.0	0.0	4.6
BrCKX6	1	12.2	5.3	3.0	3.1	4.3
BrCKX7	1	3.5	2.3	-1.6	-0.8	-0.7

Figure C

	Difference (TDZ-K - Control)					
	Days after treatment					
Gene	0 d	1 d	2 d	4 d	8 d	16 d
BrIPT2	0	13.5	7.4	4.1	6.1	2.5
BrIPT3	0	8.0	10.5	9.2	10.0	2.6
BrCKX3	0	71.6	17.9	13.6	11.7	8.8
BrCKX6	0	18.1	9.8	8.4	11.0	6.2
BrCKX7	0	6.6	12.0	7.2	7.8	1.7

Figure 5.13 Heat map of the relative expression in RCBr leaves compared to 0 d in the control and TDZ-K-treated plants. A: Heat map of control plants, B: heat map of plants following 50 µM TDZ-K treatment, C: the difference in expression between the TDZ-K treatment (B) and the control (A), with treatment minus the control presented. 0 d was defined as the appearance of true leaves and the time point prior to treatment. Leaves were acquired at this time point, followed by an application of TDZ-K 2 h after leaf acquisition, then subsequently at 2, 4, 8 and 16 days after treatment. The relative expression is represented by a heat map in the legend. Treatments were applied to the whole plants. See section **5.2.9** for more details.

Heat map of relative fold change (with 0 d reference)								
< -25	< -10 to -25	< -5 to -10	< -2 to -5	0.0	> 2 to 5	> 5 to 10	> 10 to 25	> 25

Figure A	**Control**					
	Days after treatment					
Gene	0 d	1 d	2 d	4 d	8 d	16 d
BrIPT1	1	-6.1	-11.4	-39.1	-36.9	-21.3
BrIPT2	1	-8.1	-6.0	-6.6	-5.3	-1.3
BrIPT3	1	-6.7	-10.2	-10.1	-10.0	-1.8
BrCKX3	1	-16.5	-13.9	-13.6	-11.7	-4.2
BrCKX6	1	-6.0	-4.5	-5.4	-8.0	-1.9
BrCKX7	1	-3.1	-9.7	-8.8	-8.6	-2.4

Figure B	**High N**					
	Days after treatment					
Gene	0 d	1 d	2 d	4 d	8 d	16 d
BrIPT1	1	-15.7	-8.3	-7.9	-21.6	-18.8
BrIPT2	1	-10.3	-5.7	-2.6	-2.1	-1.4
BrIPT3	1	-4.9	-5.8	-3.6	-1.0	1.6
BrCKX3	1	-22.3	-7.7	-8.9	-2.7	-0.6
BrCKX6	1	-6.3	-3.1	-4.1	-0.9	1.4
BrCKX7	1	-12.3	-4.3	-8.2	-2.5	-2.3

Figure C	**Difference (High N - Control)**					
	Days after treatment					
Gene	0 d	1 d	2 d	4 d	8 d	16 d
BrIPT1	0	-9.6	3.1	31.2	15.3	2.5
BrIPT2	0	-2.2	0.3	4.0	3.2	-0.1
BrIPT3	0	1.8	4.4	6.5	9.1	3.3
BrCKX3	0	-5.8	6.2	4.7	9.0	3.6
BrCKX6	0	-0.3	1.4	1.3	7.1	3.3
BrCKX7	0	-9.3	5.4	0.6	6.1	0.2

Figure 5.14 Heat map of the relative expression in RCBr leaves compared to 0 d in the control and high N-treated plants. A: Heat map of control plants, B: heat map of plants treated with high N (10 mM KNO_3 up from 1 mM), C: the difference in expression between the high N treatment (B) and the control (A), with treatment minus the control presented. 0 d was defined as the appearance of true leaves and the time point prior to treatment. Leaves were acquired at this time point, followed by High N 2 h after leaf acquisition, then subsequently at 2, 4, 8 and 16 days after treatment. The relative expression is represented by a heat map in the legend. Treatments were applied to the whole plants. See section **5.2.9** for more details.

Heat map of relative fold change (with 0 d reference)								
< -25	< -10 to -25	< -5 to -10	< -2 to -5	0.0	> 2 to 5	> 5 to 10	> 10 to 25	> 25

5.3.8 Gene expression in rapid cycling *Brassica rapa* during flowering and following CPPU treatments

The motive for measuring the expression of cytokinin genes during flowering and following CPPU treatment during flowering was due to preliminary growth room experiments which found an effect of CPPU on flowering. In RCBr leaf samples taken during flowering when leaves were still expanding, generally there was little expression change over the 24 hour period measured (**Figure 5.15**). A moderate increase in expression, relative to the baseline expression, is evident at 3 h for *BrIPT3* and at 24 h for *BrCKX6*. Following CPPU application, there was a reduced downregulation (based on the 0 d expression level) in the expression of *BrIPT3* at 6 and 24 h following treatment (**Figure 5.16B**) in comparison to the control (**Figure 5.16A** and **5.16C**).

	Hours from mid-flowering (20 d)		
Gene	3 h	6 h	24 h
BrIPT2	2	2.2	2.1
BrIPT3	5.9	2.2	1.6
BrCKX6	1.6	1.4	2.7
BrCKX7	2.3	2.2	1.7

Heat map of relative fold change (relative to the highest expression) control only					
0 to 2.5	> 2.5 to 5	> 5 to 10	> 10 to 25	> 25 to 50	> 50

Figure 5.15 Heat map of the relative expression of genes in leaves of RCBr plants relative to the baseline expression in control plants. Samples were taken 3 to 24 h at 20 days after sowing during flowering, where leaves were fully expanded and prior to senescence. The relative expression is represented by a heat map in the legend. See section **5.2.9** for more details.

Figure A

	Control		
	Hours after treatment at flowering		
Gene	**3 h**	**6 h**	**24 h**
BrIPT2	1	0.6	0.4
BrIPT3	1	-2.1	-4.2
BrCKX6	1	-0.2	1.6
BrCKX7	1	-0.5	-0.7

Figure B

	CPPU		
	Hours after treatment at flowering		
Gene	**3 h**	**6 h**	**24 h**
BrIPT2	1	0.1	0.3
BrIPT3	1	-0.3	-0.6
BrCKX6	1	-1.0	1.7
BrCKX7	1	-0.3	-0.2

Figure C

	Difference (CPPU - Control)		
	Hours after treatment at flowering		
Gene	**3 h**	**6 h**	**24 h**
BrIPT2	0	-0.5	-0.1
BrIPT3	0	1.8	3.6
BrCKX6	0	-0.8	0.2
BrCKX7	0	0.2	0.5

Heat map of relative fold change in respect to 3 h								
< -25	< -10 to -25	< -5 to -10	< -2 to -5	0.0	> 2 to 5	> 5 to 10	> 10 to 25	> 25

Figure 5.16 Heat map of the relative expression in RCBr leaves compared to 3 h in the control and CPPU-treated plants. A: Heat map of control plants, B: heat map of plants treated with 100 µM CPPU, C: the difference in expression between the CPPU treatment (B) and the control (A), with treatment minus the control presented. Samples were taken 3 to 24 h after treatment at 20 days after sowing during flowering, where leaves were fully expanded and prior to senescence. The relative expression is represented by a heat map in the legend. See section **5.2.9** for more details.

5.4 Discussion

5.4.1 LC-MS/MS

The LC-MS/MS analysis showed that *t*Z type cytokinins predominate at 4 daa in wheat and barley grain (**Table 5.3** and **5.4**), an observation that aligns with the high levels of cytokinin observed in dwarf wheat (Banowetz *et al*., 1999b), Stephens wheat (Banowetz *et al*., 1999a), Kopara wheat (Jameson *et al*., 1982), Chinese spring wheat grains (Morris *et al*., 1993), and in other cereals including maize (Rijavec *et al*., 2009). It is important to note that the proper detection of *c*Z was not possible in these older studies. Previous reports of low iP in wheat (Banowetz *et al*., 1999a; 1999b) and DHZ types (Jameson *et al*., 1982; Morris *et al*., 1993) are also confirmed by the LC-MS/MS analysis here. In contrast, there are reports of high levels of DHZ (Banowetz *et al*., 1999a; 1999b), and a lack of a *t*Z accumulation in barley early in grain development (Galuszka *et al*., 2004), although this latter study may have missed the transient increase in *t*Z, which is known to quickly return to baseline levels within days in wheat (Jameson *et al*., 1982; Banowetz *et al*., 1999b). With barley, there are reports of an elevation of cytokinin in grain development, with sustained increases correlating with greater grain mass (Michael and Seiler-Kelbitsch, 1972). More recently, Powell *et al*. (2013) identified *c*Z as the predominant form early in kernel development, with *t*Z predominating later. The elevation of *c*Z in barley was likely missed in the LC-MS/MS analysis reported here.

There were no significant differences in the overall pool of cytokinins or in the concentration of *t*Z and *t*ZR between any of the treatments and controls (**Tables 5.3** and **5.4**). This contrasts with experiments with CKX-inhibiting TDZ (Nagar *et al*., 2015) or when cytokinin was applied directly (Banowetz *et al*., 1999a), which resulted in increases in endogenous cytokinins. This suggests that single spray applications of INCYDE and CPPU were insufficient to alter cytokinin levels, potentially because *t*Z levels were already very high and any manipulation of cytokinin (through inhibiting CKX activity) might not noticeably change cytokinin levels.

Despite wheat and barley being closely related, there were significant differences between the cytokinin types in wheat and barley. Wheat had a strong preference for accumulating *t*Z9G (**Table 5.3**), while barley accumulated more *c*ZOG, *c*ZROG and *t*ZRMP, and had a greater concentration of *t*ZR (**Table 5.4**). The accumulation of storage cytokinins *c*ZOG and *c*ZROG at 4 daa in both wheat and barley aligns with the accumulation of *O*-glucosides previously reported in grains post-anthesis (Jameson *et al*., 1982), but that bioassay work did not discriminate between *trans* and *cis*-zeatin *O*-

glucosides. However, strong *cZOG1* and *2* expression was reported post-anthesis in Song *et al.* (2012). This observation also provides some explanation for the high levels of *c*Z reported early in barley development (Powell *et al*., 2013). Since *O*-glucosides are able to be rapidly reconverted to active forms, this accumulation of *c*ZOG allows for an ongoing supply of active form *c*Z (Werbrouck *et al*., 1996), ensuring that cytokinin-dependent processes continue over this critical period in development. This accumulation of *c*ZOG appears to be enhanced in barley grains following INCYDE treatment. INCYDE has been shown to enhance *O*-glucoside accumulation in the aerial parts of "Williams" banana plantlets when applied with BA (in comparison to BA alone) (Aremu *et al*., 2012). *O*-glucosides are resistant to N^6-side chain cleavage by CKX (Mok and Mok, 2001; Galuszka *et al*., 2007) and INCYDE might, through inhibiting CKX, be further enabling an accumulation of *c*ZOG. With strong increases in specific CKXs at this period in development (Song *et al*., 2012), having an increased presence of CKX-resistant *O*-glucosides, might be advantageous and give treated plants a sustained access to a greater pool of CKs which can be activated. These changes in barley following INCYDE treatment could also coincide with the retention of chlorophyll measured in barley pot trials (**Table 4.16**).

The N^9-glucoside *t*Z9G, which leads to irreversible inactivation of free cytokinins (Mok and Mok, 2001; Sakakibara, 2006) accumulated in barley and wheat. This aligns with observations of *N*-glucosyltransferases showing strong expression during seed development (Wang *et al*., 2011b). Although there was a higher level of *t*Z9G following INCYDE treatment of barley, this difference was not statistically significant (**Table 5.4**). This contrasts with observations elsewhere, where an INCYDE-induced enhancement of *t*Z9G was observed in "Williams" banana plantlets when INCYDE was applied in presence of mT (compared to mT) in aerial parts (Aremu *et al*., 2012).

Even though statistically significant differences were measured between treatments and controls in barley grains for *c*ZR and DHZ types (**Table 5.4**) each of these differences were with cytokinins present at low concentrations compared to the bioactive *t*Z. These relatively small differences are therefore unlikely to be of any real, physiologically meaningful.

5.4.2 RT-qPCR analyses

5.4.2.1 Wheat *TaIPT* and *TaCKX* expression

Members of gene families *TaIPT* and *TaCKX* showed spatio-temporally differential expression patterns (**Figure 5.6**), an observation consistent with previous expression studies with wheat (Song *et*

al., 2010; 2012). The high levels of *TaIPT3, 5* and *7* in grain tissue immediately post-anthesis (compared to baseline expression) (**Figure 5.6**) are potentially responsible for the high levels of *t*Z 4 daa (**Tables 5.3** and **5.4**), and the high cytokinin levels post-anthesis shown in other wheat cultivars (Jameson *et al*., 1982; Morris *et al*., 1993; Banowetz *et al*., 1999b). The presence of strong *TaIPT* expression indicates that a significant proportion of cytokinin used in developing grain is biosynthesised locally rather than supplied from other tissues. The strong increase in *TaIPT5* expression in grain post-anthesis was also observed in Song *et al.* (2012) and in the reproductive organs of barley with orthologue *HvIPT5* (Mrízová *et al*., 2013). Although *TaIPT2* expression modestly increased at 4 to 14 daa in grain, it likely has a role in grain development, as orthologue *ZmIPT2* was expressed in the endosperm, basal endosperm transfer layer and developing embryo of maize, and was implicated in cell division and the establishment of sink strength (Brugière *et al*., 2008).

The modest expression of *TaCKX1* and *3* after 4 daa in grain (**Figure 5.6**) coincides with a rapid decline in cytokinin reported following an initial increase post-anthesis in wheat (Jameson *et al*., 1982; Banowetz *et al*., 1999b). In contrast to the gene expression profile described by Song *et al*. (2012), however, *TaCKX1* did not show consistent strong expression post-anthesis. The trials carried out with Song *et al*. (2012) were carried out in Lincoln, Canterbury (not far from the field trials carried out this project) in a nursery plot, and just as was the case with the field trials in this project, these plots were subject to prevailing climatic conditions. The cultivar used in Song *et al*. (2012): Equinox, was not used in this research project, however. *TaCKX1* is known to have a crucial role early in grain development, with evidence of high levels of expression and/or activity of *CKX1* orthologues in the seed development of cereals (Brugière *et al*., 2003; Galuszka *et al*., 2004; 2005; Šmehilová *et al*., 2009; Zalewski *et al*., 2010; 2014), and with *CKX1* expression correlating with cytokinin levels (Brugière *et al*., 2003). The importance of *CKX1* in yield determination has been highlighted with RNAi experiments targeting *HvCKX1*, which resulted in increases in yield (Zalewski *et al*., 2010). Despite the modest levels of expression of *TaCKX1* measured, it is evidently an important biotechnological target for altering yield. *CKX2* is also known to have an important role in grain development and spike morphogenesis in wheat (Zhang *et al*., 2011; Song *et al*., 2012) and to be involved in yield determination in barley (Zalewski *et al*., 2012). However, while expression was detected in the flag leaf, there was minimal change in expression during grain development. The strong expression of *TaIPT7* during wheat leaf development (**Figure 5.6**) indicates the strong presence of locally-produced cytokinins at this point in development, and given the timing of this production is potentially connected to the grain and reproductive development of the plant.

The sustained upregulation of *TaCKX8* at and after 4 days (based on the 0 d expression level) in wheat leaves following INCYDE treatment (**Figure 5.7B**) compared to the control suggests a feedback response to normalise endogenous cytokinin levels, which might have been enhanced following an inhibition of CKX (Zatloukal *et al*., 2008). This observation appears to implicate *TaCKX8* as a key response gene to INCYDE treatment. The downregulation of *TaIPT*2 following INCYDE treatment aligns with previous research showing an *IPT* downregulation in response to an enhancement in cytokinin in maize leaves (Vyroubalová *et al*., 2009). This *TaIPT* downregulation might also be part of a feedback response to normalise cytokinin levels.

Somewhat unexpectedly, there was reduced upregulation and even downregulation of other *TaCKX*s 4 days after INCYDE treatment compared to the control, with the exception of *TaCKX8* (**Figure 5.7**). In other studies there was evidence that, following exogenous CK application, there is both the up and downregulation of *CKX*s (Vyroubalová *et al*., 2009, Liu *et al*., 2013). Individual CKXs can also have different substrate preferences, they are differentially regulated and expressed in tissue and subcellular locations, and CKX activity is regulated by a range of factors including local pH (Bilyeu *et al*., 2001; Galuszka *et al*., 2001; 2004; 2007; Frébort *et al*., 2002; Werner *et al*., 2003, 2006; Hirose *et al*., 2008; Pertry *et al*., 2009; Kowalska *et al*., 2010; Zalabák *et al*., 2014). Therefore changes in the up and downregulation of *TaCKX*s following PGR treatments was not unexpected.

There are no current reports of TDZ-K affecting cytokinin homeostasis and regulation, and TDZ-K is stated not to inhibit CKX (J. Nisler, personal communication, August 28, 2017). There is evidence that TDZ-K shows cytokinin activity through its retention of chlorophyll in detached leaf assays with wheat and barley, its dose-dependent promotion of growth in tobacco callus and its property of having an EC_{50} comparable to *t*Z with *Amaranthus* bioassays (United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript). The differences in the expression of *TaIPT*s and *TaCKX*s compared to the control in both grain and leaf tissues following TDZ-K treatment (**Figures 5.8** and **5.9**) suggests that TDZ-K did indeed influence cytokinin regulation in wheat. Given the strong link between cytokinins and senescence or senescence-associated processes (Clarke *et al*., 1994; Noodén *et al*., 1997; Jordi *et al*., 2000; McCabe *et al*., 2001), it is very possible that the effect of TDZ-K on cytokinin-associated genes is through the known effect of TDZ-K on photosynthetic complexes (J. Nisler, personal communication, August 28, 2017; United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript), or alternatively, by some other mechanism.

Notably, there was a stronger, more widespread effect of TDZ-K on *TaIPT* expression in the grain tissue (**Figure 5.9**) compared to the leaf (**Figure 5.8**), and this suggests that reproductive tissue was more sensitive to PGR-induced disruption of cytokinin homeostasis or that cytokinin production was more inducible in grain tissue at this point during development.

Following CPPU treatment, there was a sustained upregulation of *TaCKX3* and *TaCKX4* in wheat grains (**Figure 5.10B**) that was not observed in the control (**Figure 5.10A**). Given that CPPU inhibits CKX activity (Bilyeu *et al*., 2001), it is likely that this upregulation was a response to a resulting increase in cytokinin. This observation aligns with reports of an upregulation of CKX activity and/or expression following an enhancement in cytokinin (Motyka *et al*., 1996; 2003; Brugière *et al*., 2003; Blagoeva *et al*., 2004; Hirose *et al*., 2008; Vyroubalová *et al*., 2009). Orthologue *HvCKX4* has been previously identified in developing barley kernels for its high levels of expression, and was suggested as a suitable target for increasing yield (Zalewski *et al*., 2014). The strong upregulation in *TaCKX4* might help explain the decreases in yield measured in the field trial (**Table 3.11**) and pot trials (**Table 4.6**) after targeting anthesis with CPPU, and this observation would be consistent with Ashikari *et al*. (2005), where an overexpression of *OsCKX2* in rice corresponded with a reduction in yield.

The lack of (or reduced) downregulation of *TaIPT3*, *5* and *7*, following treatment (**Figure 5.10B**) compared to the control (**Figure 5.10A**) in grain at or after 2 days following CPPU treatment appears to correlate with upregulation observed in *TaCKX3* and *4* (**Figures 5.10B** and **5.10C**). CPPU inhibits CKX activity, which might have led to a feedback response where *TaCKX* was upregulated, reducing endogenous cytokinin and subsequently leading to a reduced downregulation (or upregulation) of *TaIPT*s (**Figure 5.10B**) to normalise cytokinin levels. This observation partly aligns with the feedback model proposed for the response of RCBr to INCYDE (section **2.4.3**). An upregulation of *IPT*s has also been observed when cytokinin levels were enhanced by exogenous application (Vyroubalová *et al*., 2009) or following *IPT*-overexpression (Hoth *et al*., 2003). The reduced upregulation of *TaIPT3* immediately following treatment (1 d) (**Figure 5.10B**) compared to the control (**Figure 5.10A**), and the later downregulation in the expression of *TaCKX10* (at 14 days), following the upregulation of *TaCKX3* and *4*, might also be a part of this mechanism responsible for the normalisation of cytokinin levels at different time points following CPPU treatment.

Despite the evidence of a gene expression response to INCYDE, TDZ-K and CPPU application, this did not translate into significant changes in the concentration of active cytokinins in the LC-MS/MS analyses in wheat (**Tables 5.3** and **5.4**). The relative gene expression changes observed with several

*TaIPT*s and *TaCKX*s might be too modest, or there might be a feedback response that prevented any significant change in the concentration of active cytokinins. The endogenous cytokinin concentrations may have actually changed by small quantities, but it might be difficult to observe this due to the relatively high concentration of some cytokinins. Even with the evidence of a gene expression response here, it is possible that some of the gene expression response to CKX-inhibiting INCYDE or CPPU was missed because it may have occurred over a very short time frame, such as within 24 hours. Indeed, there are reports of increases in *CKX* expression and/or activity within hours following cytokinin application (Vyroubalová *et al*., 2009; Liu *et al*., 2013).

5.4.2.2 Wheat *TaZOG2* and *TaGLU1a* expression

TaZOG2 expression appears stronger in wheat leaves in comparison to grains (**Figure 5.6**), although given that these samples were analysed separately, it is difficult to compare the expression of each sample. In comparison, Song *et al.* (2012) observed low levels of expression of *TaZOG*s in both leaf and grain tissue. The low levels of *TaZOG2* coincides with the relatively low levels of *t*ZOG observed in wheat grains (**Table 5.3**). In contrast, Song *et al.* (2012) reported high levels of expression of some *TacZOG*s, which might explain the comparatively greater levels of *c*ZOG present in wheat grains at 4 daa.

In the wheat flag leaf, the lack of a strong downregulation (based on the 0 d expression level) response of *TaZOG2*, at 7 days following treatment with INCYDE (**Figure 5.7B**) or TDZ-K (**Figure 5.8B**) compared to their respective controls (**Figures 5.7A**, **5.7C**, **5.8A** and **5.8C**) suggests that the *O*-glucosylation of *t*Z is a longer term response to PGR-induced changes in cytokinin homeostasis.

TaGLU1a, which produces *β*-glucosidases which deconjugate *O*-glucosides, releasing the free base forms, has high levels of expression in both grain and leaf samples (**Figure 5.6**). The expression patterns also fit well with *TaGLU1-1* (equivalent to *TaGLU1a*) in Song *et al.* (2012). The high levels of expression could offer some explanation for the high levels of *t*Z in wheat grain (**Table 5.3**) and increases in cytokinin observed in other studies (Jameson *et al*., 1982; Morris *et al*., 1993; Banowetz *et al*., 1999a; Banowetz *et al*., 1999b).

The lower levels of upregulation of *TaGLU1a* in wheat leaves immediately following INCYDE treatment (**Figure 5.7B**) compared to the control (**Figures 5.7A** and **5.7C**) suggests that potentially there is a short term reduction in the reactivation of cytokinin, as a result of an increase in cytokinin

following inhibition of CKX by INCYDE (Zatloukal *et al*., 2008). The subsequent sustained upregulation of *TaGLU1a* following INCYDE treatment suggests a potential correction of this disruption in cytokinin reactivation at a later time point.

Likewise, the downregulation and reduced upregulation of expression of *TaGLU1a* in leaves (at 1 and 4 days after treatment) following TDZ-K treatment (**Figure 5.8B**) compared to the strong upregulation at these time points in the control (**Figures 5.8A** and **5.8C**) provides further evidence that this compound, by some unknown mechanism, is able to modify regulatory components of cytokinin homeostasis. Experiments where genes encoding *β*-glucosidases have been overexpressed (Kiran *et al*., 2006; 2012; Nguyen *et al*., 2015), have been observed to alter the phenotype of tobacco plants by increasing the biomass (Nguyen *et al*., 2015) and creating taller plants (Jung *et al*., 2013). The reduced expression of *TaGLU1a* following TDZ-K treatment could explain the reduction in stem growth (in the diameter) in wheat drought trials (**Table 4.9**).

In grain tissue, TDZ-K and CPPU appeared to have a little effect on *TaGLU1a* expression immediately after treatment (1 day) (**Figures 5.9B** and **5.10B**), suggesting that the active and conjugated cytokinins were tightly controlled immediately post-anthesis. The lack of expression change in the grain helps explain why there were not any significant changes in developing grain in the pool of active or conjugated cytokinins following treatment (**Table 5.3**), and this could help explain the lack of effect of each compound on the yield in field and pot trials.

5.4.2.3 Wheat *TaRRA4* and *TaCWINV* expression

There was little change in the expression of *TaRRA4* in the flag leaf or grain between 7 and 14 days after anthesis (**Figure 5.6**). In Song *et al*. (2012), gene expression of *TaRRA4* was also modest in grain, but in flag leaves *TaRRA4* showed strong expression between 7 and 14 daa, suggesting that an increase in expression was missed as leaf samples were only acquired up to 7 days after anthesis.

Given that response regulator activity and expression are the ultimate indicators of changes in endogenous cytokinin levels (D'Agostino *et al*., 2000; Che *et al*., 2002; Kiba *et al*., 2002; Rashotte *et al*., 2003), the change in the expression pattern of *TaRRA4* following each PGR treatment (**Figures 5.7C**, **5.8C**, **5.9C** and **5.10C**) confirms that each PGR does in fact affect endogenous cytokinin levels and cytokinin homeostasis. The transcription levels of *RRA*s have been suggested to correlate with cytokinin levels (Imamura *et al*., 1999; Jain *et al*., 2006; Kurakawa *et al*., 2007), although evidence of

correlations between the expression of *TaIPT*s and *TaRRA*s have been shown more recently to only be modest (Song *et al*., 2012). Modest correlations with *TaIPT* expression are evident in untreated grain (**Figure 5.6**), with the highest levels of expression (compared to baseline expression) at 0 to 1 daa for *TaRRA4* and for most *TaIPT*s. Correlations following PGR treatment seem more clear, there appears to be a correlation between the downregulation of *TaIPT*s and *TaRRA4* (based on the 0 d expression level) in flag leaves (**Figures 5.8B** and **5.8C**), while in grain there is a similar change in expression pattern with both *TaIPT*s and *TaRRA4* both upregulating (relative to the control expression levels) following both TDZ-K (**Figure 5.9C**) and CPPU (**Figure 5.10C**) treatments. This suggests that *TaRRA4* expression appeared to correlate with cytokinin biosynthesis.

TaCWINV1 and *2* are differentially expressed in grains (**Figure 5.6**). The increase in *TaCWINV1* expression between 2 and 7 daa is in agreement with expression studies of orthologue *ZmCWINV2*, which increased from 0 to 12 days after pollination (Carlson and Chourey, 1999). Given the role of *CWINV*s in determining sink strength and grain weight (LeClere *et al*., 2010), the modest upregulation (relative to the control expression levels) of *TaCWINV2* following INCYDE treatment (**Figure 5.7C**), the modest upregulation following CPPU treatment (**Figure 5.10C**), and following TDZ-K treatment in the grain (**Figure 5.9C**) suggests that through altering cytokinin homeostasis and/or senescence, these compounds affect sink source dynamics in wheat. The differential response of *TaCWINV1* to treatments provides further evidence for the differential regulation of each *TaCWINV*.

5.4.2.4 Rapid cycling *Brassica rapa*

Expression studies with RCBr revealed temporal-specific expression patterns (**Figure 5.11**). The expression of *BrIPT*s and *BrCKX*s (compared to baseline expression) appears to be in parallel, with the strongest expression of members of both gene families in the young leaves at 0 daa, with a parallel reduction in expression between 1 and 8 daa when leaves were expanding. These expression patterns are in agreement with RCBr expression studies of O'Keefe *et al*. (2011), and suggest that there were high levels of cytokinin activity earlier in development. The strong expression of *BrIPT1* implicates this gene family member in having a key role in leaf development. Conversely, in O'Keefe *et al*. (2011), *BrIPT3* had the strongest expression relative to other *BrIPT*s, while in *B. napus*, *BnIPT1* had very low expression in leaves (Song *et al*., 2015). In comparison to *BrIPT1*, *BrIPT2* expression was modest and relatively stable. This is consistent with knowledge of *BrIPT2* being a tRNA isopentenyltransferase, that has been shown to be constitutively and ubiquitously expressed at low levels (Liu *et al*., 2013; Song *et al*., 2015). The strong expression of *BrIPT3* (O'Keefe *et al*., 2011)

and *BnIPT3* (Song *et al*., 2015) in mature leaves was not observed, likely because samples were not acquired late enough in development. *BrCKX3* showed the strongest expression levels of *BrCKX*s at leaf appearance (0 d), which is in agreement with recent expression studies with RCBr (O'Keefe *et al*., 2011). The expression level of *BrCKX6* was low in contrast to *B. napus* (Song *et al*., 2015) and Chinese cabbage (Liu *et al*., 2013), while the relatively stable and modest expression level of *BrCKX7* was more in line with observations in other *Brassica* spp. (Liu *et al*., 2013; Song *et al*., 2015).

The strong expression of *BrCKX*s early in development (**Figure 5.11**), combined with research where arabidopsis *ckx* mutants have enhanced cytokinin in reproductive meristems and consequentially yield (Bartrina *et al*., 2011), indicates that targeting RCBr plants with CKX-inhibiting INCYDE early in development, was an appropriate approach in the growth room experiments. Given that samples were acquired from the lower (older leaves), due to the insufficient size of the upper (young leaves), it is likely that higher levels of expression would have been measured in younger leaves. Nonetheless, expression patterns are still clear and evident in older leaves.

The strong, upregulation (based on the 0 d expression level) of *BrCKX3* immediately following INCYDE treatment (**Figure 5.12B**) in contrast to the strong downregulation in the control (**Figures 5.12A** and **5.12C**) indicates a feedback response to an enhancement of cytokinin, which resulted from the inhibition of CKX. Increasing endogenous cytokinin with BA treatment also resulted in an upregulation of *BrCKX3* in Chinese cabbage (Liu *et al*., 2013), and it has been observed in a number of studies that *CKX* expression and/or activity increases as a result of an enhancement of cytokinin (Chatfield and Armstrong, 1986; Kamínek and Armstrong, 1990; Motyka and Kamínek, 1990; Motyka *et al*., 1996; 2003; Brugière *et al*., 2003; Blagoeva *et al*., 2004; Hirose *et al*., 2008; Vyroubalová *et al*., 2009; Jameson and Song, 2016). This upregulation in gene family members associated with degradation appears to be systemic, with the other *BrCKX*s upregulated relative to the control (**Figure 5.12C**) at several days after treatment. This response likely normalises cytokinin levels. The further downregulation (relative to the control expression levels) of *BrIPT1* and *BrIPT3* (**Figures 5.12C**) following INCYDE treatment, is also potentially a part of this feedback response to normalise cytokinin levels, and aligns with other work where *IPT*s were downregulated in response to enhanced cytokinin from BA treatment (Miyawaki *et al*., 2004; Liu *et al*., 2013). The subsequent upregulation of *BrIPT*s at 2 to 8 days (**Figure 5.12C**) is likely a part of this normalisation of cytokinin levels, as cytokinin levels have likely been reduced within the first 24 hours following treatment. These observations provide evidence for the feedback response model used to describe the effect of INCYDE on yield in RCBr (section **2.4.3**).

The upregulation (based on 0 d expression levels) of *BrCKX*s following TDZ-K treatment (**Figure 5.13B**), compared to the downregulation of these gene family members in the control (**Figure 5.13A**), provides further evidence that this PGR has an effect on cytokinin regulation, although this response differs from that observed following INCYDE treatment, with a systemic upregulation (relative to the control expression levels) of all *BrIPT*s and *BrCKX*s immediately after treatment (**Figure 5.13C**). As was the case following INCYDE treatment, there was a strong upregulation (compared to baseline expression) of *BrCKX3* immediately following treatment (**Figure 5.13B**), which suggests that this gene plays a key role in response to disruptions to cytokinin homeostasis or cytokinin-associated processes (including senescence). This effect of TDZ-K on cytokinin aligns with evidence of TDZ-K showing cytokinin activity including chlorophyll retention and growth promotion in tobacco callus (United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript).

The further downregulation in the expression of both *BrIPT*s and *BrCKX*s within one day of increasing the concentration of nitrate from 1 to 10 mM (**Figure 5.14B**), indicates a transient adaption to high nitrate conditions. The longer term response indicates that the presence of higher concentrations of nitrate results in a reduction in the downregulation in the expression of both *BrIPT*s and *BrCKX*s, particularly of *BrIPT1*, and this observation aligns with previous studies which describe nitrate-inducible genes including *AtIPT3* (Miyawaki *et al*., 2004; Takei *et al*., 2004a).

The low levels of expression in RCBr leaves during flowering (**Figure 5.15**), aligns with the low levels of expression measured at the beginning of flowering, eight days after leaf appearance (**Figure 5.11**), while it is evident that CPPU failed to have much impact on any of the *BrIPT*s and *BrCKX*s measured (**Figure 5.16**). Given the effects of INCYDE and TDZ-K on RCBr were observed over several days following treatment, it was likely that samples were acquired over too short a period of time following CPPU treatment.

5.4.3 Summary

In summary, it is evident that *t*Z cytokinin is present in both wheat and barley grains at high levels, relative to other cytokinins in these samples, at four days after anthesis, while there were very low levels of other free base cytokinins. The PGRs had little effect on the accumulation of endogenous cytokinins with only INCYDE affecting the concentration of *O*-glucosides (*c*ZOG). This lack of change in cytokinin levels could explain why there were very few changes in yield observed in the wheat and barley field trials (Chapter 3) and pot trials (Chapter 4) following treatment at anthesis.

This lack of change in endogenous cytokinin levels was not, however, reflected in expression studies of wheat grain and flag leaf and the reasons for this disparity include the fact that these expression changes might be of little biological significance (perhaps due to feedback systems), and there are a multitude of other regulatory systems and other factors that affect cytokinin physiology which cannot be accounted for with expression studies alone. Each PGR was able to induce changes in the expression of genes associated with cytokinin, cytokinin-associated processes and metabolism, and these changes help inform an understanding of their mechanisms and effects.

Critically, the change in expression of the response regulators following each PGR (**Figures 5.7C**, **5.8C**, **5.9C** and **5.10C**), confirms that INCYDE, TDZ-K and CPPU all have an effect, even if modest, on endogenous cytokinin levels. Additionally, a correlation between the expression of response regulators (*TaRRA4*) and cytokinin biosynthesising isopentenyltransferases (*TaIPT*s) was confirmed. These findings also highlighted which genes should be the focus of future work. Primers for cell wall invertase genes (*TaCWINV*) were successfully designed and used, and showed modest expression at different stages of grain development and in response to each PGR.

The expression of genes in wheat grains and leaf samples only partly aligned with previous expression studies with wheat (Song *et al*., 2010; 2012), which were grown under similar conditions. Notably, the strong expression of *TaCKX1* and *TaIPT2* in both grain and leaf tissue post-anthesis (Song *et al*., 2012), was not observed in the expression studies reported in this Chapter, potentially because of the handling of grain samples during sorting prior to RNA extraction, which might have exposed samples to RNases, or potentially because experiments were carried out with different wheat cultivars.

Gene expression studies with RCBr aligned with previous RCBr work (O'Keefe *et al*., 2011), and indicated high levels of expression of *BrIPT*s and *BrCKX*s in young leaves, followed by a reduction in expression as leaves expanded. Following INCYDE treatment, the gene expression response provided some evidence for the INCYDE feedback model proposed following the growth room experiments (section **2.4.3**). Even though there was little evidence in pot and field trials to suggest that TDZ-K delayed senescence or enhanced yield, the gene expression studies (for both RCBr and wheat) provided evidence that, by some mechanism, TDZ-K does influence cytokinin homeostasis. This aligns with the cytokinin activity shown by its ability to inhibit senescence, its dose-dependent promotion of growth in tobacco callus and having an EC_{50} comparable to *t*Z with *Amaranthus* bioassays (Nisler *et al*., unpublished manuscript).

Although expression studies with wheat suggested an effect of CPPU on several gene families, there was little evidence to suggest CPPU significantly altered the expression of cytokinin-related genes in RCBr, although this could have been because of the limited number of time points analysed in RCBr. Conversely, in response to increasing the supply of nitrate, RCBr showed both a transient increased downregulation and longer term reduction in downregulation in the expression of *BrIPT*s and *BrCKX*s which indicated an adaption to an altered nutrient status. Ultimately, both the wheat and RCBr gene expression results provided useful information about the nature of cytokinin regulation and how various treatments and growth conditions can influence cytokinin homeostasis.

Chapter 6
Final discussion

6.1 Field and pot trials with wheat and barley

While the gene expression data support the novel PGRs INCYDE and TDZ-K as being biologically active compounds, the field and pot trial data do not support the claims that INCYDE, TDZ-K or CPPU have the capacity to increase the yield and/or biomass of wheat and barley under the conditions used in these trials. In general the effect of the PGRs were limited in both the field and pot trials, with the most common outcome being no change in components of yield or biomass following the application of the PGRs in either the field (Chapter 3) or small pots (Chapter 4). Where changes in yield were evident, these changes were decreases in the thousand grain weight. There are several possible reasons for this outcome, which will be discussed here, and these findings can inform future work and directions.

Targeting PGRs at anthesis (GS 60-69) was originally justified based on consistent evidence of increases in the content of cytokinin post-anthesis in wheat (Jameson *et al*., 1982; Morris *et al*., 1993; Banowetz *et al*., 1999a; Banowetz *et al*., 1999b) and barley (Michael and Seiler-Kelbitsch, 1972) and in the upregulation of cytokinin-associated genes in wheat (Song *et al*., 2012) and *HvCKX*s in barley anthesis as the kernel develops (Zalewski *et al*., 2014), and on experiments which indicate that cytokinin is limiting seed size, such as experiments where cytokinin was directly injected into the plant (Dietrich *et al*., 1995; Sivakumar *et al*., 2001). High levels of *t*Z-type cytokinins (compared to other cytokinin types) were confirmed in LC-MS/MS analyses of wheat and barley at four days after anthesis (**Table 5.3** and **5.4**).

With TDZ-K, trials were recently carried out by our collaborator laboratory based at Palacký University (United States Patent US 2017/0280721 A1, 2017), and these trials revealed an enhancement following a single foliar application of TDZ-K once at 25 µM before flowering, at the end of stem elongation/bud appearance in winter oilseed rape, and at head emergence in spring barley. These trials were carried out without the use of other PGRs or fungicides. These findings suggest that future experiments should focus on earlier growth stages, rather than the anthesis and early senescence that was targeted in field (Chapter 3) and pot trials (Chapter 4). In future the use of other PGRs, fungicides and pesticides should also be avoided.

Given the reported capacity of INCYDE and CPPU to inhibit CKX (Bilyeu *et al*., 2001; Zatloukal *et al*., 2008) and the high levels of *CKX* expression post-anthesis (Song *et al*., 2012; Zalewski *et al*., 2014), there was the expectation that treatments with these compounds would elevate the concentration of cytokinin in developing grains. However, aside from changes in the *O*-glucoside concentration in barley grains following INCYDE treatment (**Table 5.4**), the LC-MS/MS analyses showed that neither INCYDE, CPPU, or senescence-inhibiting TDZ-K treatments at anthesis were able to significantly change the total concentration of cytokinins or the concentration of active cytokinins in wheat or barley grains. The increase in conjugated cytokinins might suggest a regulatory response to an enhancement in active cytokinins following inhibition of CKX with INCYDE, and this in itself provides an area for future research.

Ultimately, the lack of change in the total endogenous concentration of cytokinin in barley and wheat grains provided some explanation for the lack of yield enhancement observed in the field following PGR treatments. However, each PGR had an effect on the expression of *TaIPT*s and *TaCKX*s (**Figures 5.7**, **5.8**, **5.9** and **5.10**). There are several possible reasons for this disconnect between changes in the gene expression and a lack of changes to the endogenous cytokinin levels. These include the possibility that gene expression changes might ultimately have been relatively small and there is likely a feedback response as part of this effect. There may have been rapid changes within 24 h, or small changes in the endogenous cytokinin concentrations, and these might have been difficult to observe (or statistically non-significant) due to the relatively high concentrations of some cytokinins.

Members of gene families *TaIPT*, *TaCKX*, *TaCWINV*s and genes *TaGLU1a*, *TaRRA4* and *TaZOG2* were differentially upregulated or downregulated in response to each PGR, and differentially between different tissues (leaf and grain) in response to TDZ-K. Critically, the changes in the expression of *TaRRA4* following each PGR, even if modest in some cases, confirms that each PGR is able to modify endogenous cytokinin levels. With TDZ-K, this aligns with the cytokinin activity previously demonstrated, including the retention of chlorophyll in detached leaf assays, its dose-dependent promotion of growth in tobacco callus, having an EC_{50} comparable to *t*Z with Amaranthus bioassays, and enhancing yield in barley and winter oilseed rape (J. Nisler, personal communication, August 28, 2017; United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript). Conversely, TDZ-K also exhibits activity that is distinctly different from cytokinin, including not showing a biphasic response when applied at high concentrations on tobacco callus growth, and not inhibiting root growth when applied at 100 nM to wheat and arabidopsis (United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript).

There appeared to be feedback response following CKX-inhibition by INCYDE, with *TaCKX8* upregulated and *TaIPT2* downregulated following application to wheat leaves. While in grain tissue following CPPU application, an upregulation of *TaCKX3* and *4* appeared to be a part of this feedback. These gene families warrant further expression research to determine the role they might play in this feedback. Given other research which suggests that antisense targeting of *OsCKX2* in rice resulted in a decrease in inflorescence meristem-expressed *OsCKX2*, an enhancement of cytokinins and an increase in yield (Ashikari *et al*., 2005), and experiments where barley grain-expressed *CKX*s were silenced by RNAi, which enhanced yield (Zalewski *et al*., 2010; 2012; 2014), the *TaCKX*s identified in these gene expression analyses could be important transgenic targets for yield enhancement.

There was a lack of yield enhancement when INCYDE was targeted at anthesis. In contrast, there was a non-significant increase in TGW at position 1 to 2 in large stems in wheat (which was non-significant with the conservative Tukey HSD test) (**Table 3.7**). Additionally, there are claims of yield enhancements by collaborators (personal communication, February 24, 2017) and alterations in the yield and/or growth reported following the targeting of a range of earlier growth stages with cytokinin, including pre-sowing, following sowing and during tillering (Jafar *et al*., 2012; Afzal *et al*., 2013; Criado *et al*., 2009; Yasmeen *et al*., 2013). Collectively, these observations make the case for targeting earlier growth stages in future trials.

Another reason for the lack of yield enhancement, might have been the selection of Yates Sprayfix as a surfactant for the field and pot trials. While Yates Sprayfix is used broadly in a range of horticultural contexts, its composition is unknown (i.e. not revealed), and it has a significant effect on the retention and uptake of each PGR. Future field trial experiments should explore the effect of different surfactants on the effect and efficacy of these PGRs.

Targeting 30 µM of CPPU at anthesis (GS 61, 65) caused a decrease in wheat TGW both in field (**Table 3.11**) and pot trials (**Table 4.6**), while 100 µM CPPU caused a decrease in the TGW in barley drought trials (**Table 4.11**). Likewise, INCYDE targeted at anthesis in barley resulted in a decline in TGW in both field (**Table 3.13**) and in pot trials (**Table 4.7**). While this suggests that some of the effects on yield were consistent across different environments and independent of the presence of stress or optimised conditions, this was not the case with chlorophyll retention following INCYDE treatment, where INCYDE resulted in a retention of chlorophyll in barley leaves when targeting both GS 51 (**Table 4.15**) and GS 61 (**Table 4.16**) in pots, but not in field trials (**Table 3.15**). This suggests that the presence of stress has some role in the effect of INCYDE. The decline in yield following

INCYDE and CPPU application may be a result of a feedback response, such as an upregulation of *TaCKX* expression, which would have reduced endogenous cytokinin content. As cytokinin analysis of only one time point was available, changes in the endogenous cytokinin content may have been missed.

The yield enhancement observed in the controlled growth room trials with RCBr (**Table 2.3**) could not be replicated in the field with cereals. Although this could be due, in part, to the physiological differences between dicots and monocots, and the growth stages targeted in the cereals, the presence of environmental effects and stressors is also likely to play a role in this disparity. There are examples of the difficulty in attempting to repeat results from controlled experiments in a field trial. For example, BA treatment under a controlled hydroponic setup in glasshouses enhanced yield in soybeans, but this could not be repeated in the field (Nagel *et al*., 2001).

INCYDE, which inhibits CKX activity (Zatloukal *et al*., 2008), delayed senescence presumably due to an increase in endogenous cytokinin, which would affect chlorophyll content in a manner similar to that observed in exogenous cytokinin application experiments (Clarke *et al*., 1994; Noodén *et al*., 1997) or experiments with ectopic *IPT*-overexpression (Guo and Gan, 2014, and references therein). On the other hand, although these trials confirmed the fact that TDZ-K did not inhibit root length in wheat (**Table 3.6**) (United States Patent US 2017/0280721 A1, 2017) in contrast to TDZ, these trials failed to support the claims of TDZ-K being able to delay in detached leaf senescence assays (when applied between 1 and 100 µM) (personal communication, August 28, 2017; United States Patent US 2017/0280721 A1, 2017; Nisler *et al*., unpublished manuscript).

The inconsistent responses between controlled growth room conditions, optimised field trials, and stressed pot trials highlighted the complex interaction between stress, senescence, cytokinin and source-sink relations, and the multitude of processes associated with each. Therefore, given these results and the importance and complexity of cytokinin in the stress response (O'Brien and Benková, 2013; Zwack and Rashotte, 2015), future work will need to determine the contributing role that individual stressors can play in the efficacy of PGRs, for example for drought experiments, PEG could be applied under controlled laboratory conditions.

Targeting CKX activity was suggested to be a better approach to manipulating cytokinin levels in comparison to applying cytokinin directly, such as with BA or kinetin (Gemrotová *et al*., 2013; Nisler *et al*., 2016). Even in transgenic experiments, targeting *CKX* was identified as a good approach for

manipulating cytokinin as *CKX* was suggested to be a 'softer regulator' compared to *IPT* (Zhao *et al*., 2015). The case for using compounds that target CKX as opposed to direct cytokinin application, was further highlighted by a multitude of experiments showing the complexity and often inconsistencies associated with direct cytokinin application (Koprna *et al*., 2016), and provided an impetus for investigating the CKX-inhibiting PGRs CPPU and novel compound INCYDE. However, the field and pot trials (Chapter 3 and 4) indicated that manipulating cytokinin by CKX inhibition failed to enhance yield, and sometimes resulted in inconsistent effects on yield and growth under optimal and sub-optimal conditions. Indeed, these results indicated a complex response which aligns with the pleiotropic nature of cytokinins (Jameson and Song, 2016), made all the more complex by the broad involvement of cytokinins in a range of processes across the plant (Miyawaki *et al*., 2004; Bartrina *et al*., 2011; Brenner and Schmülling, 2012) which includes crosstalk with other plant hormones (Koprna *et al*., 2016).

Even when using transgenic approaches, significant care was required to carefully manipulate cytokinin levels. When using *IPT*-overexpression to enhance cytokinin content, transgenic plants often developed undesirable and abnormal characteristics due to plant-wide responses to cytokinin overproduction and redistribution via the plant vascular tissue (Cowan *et al*., 2005). This approach required highly specific and controlled senescence-inducible promoters (P_{SAG12} or P_{SARK}) fused to an *IPT* and controlled in an autoregulatory manner in order to produce desirable growth and yield effects (Guo and Gan, 2014, and references therein). Given the care required with transgenics to modestly and precisely manipulate cytokinin, it is clear that there were going to be difficulties and complexities inherent in the significantly less precise approach of exogenously applying treatments to whole plants.

6.2 Rapid cycling *Brassica rapa*

The growth room experiments (Chapter 2) showed that INCYDE increased seed yield in RCBr when applied four times prior to flowering (**Table 2.3**), but only under specific nitrate conditions (5 mM KNO_3). The reduction of chlorophyll measured in the lower leaves following INCYDE treatment (**Table 2.7**) could be indicating that the enhanced yield (stronger sink) increased the drain on the source leaves. This narrow effect contrasted with preliminary experiments (unpublished data, Palacký University) which showed widespread change in growth and yield.

The reasons for this disparity in effect were discussed in section **2.4.2**. These include the use of the surfactant Tween 20 in comparison to Silwet 806 used in the Palacký University experiments, which

likely affected uptake of the PGR, although preliminary replicates with Silwet L-77 did not indicate a broad response on growth and yield traits (section **5.2.1.3**). Another significant reason includes the Palacký University experiments using field conditions with winter rapeseed, although little information is given on these specifics of these conditions, this would introduce additional environmental factors. Another possibility is the use of different species, the winter rapeseed (*Brassica napus* L.) and arabidopsis, both of which would have grown to a larger size than RCBr.

The requirement for multiple applications led to a feedback model to help explain the observation. Changes in gene expression in RCBr following INCYDE treatment (**Figures 5.12B** and **5.12C**) provided supporting evidence for the model, as the expression aligned with the observed effects of INCYDE on yield of RCBr. The model indicates that following INCYDE treatment, CKX activity is inhibited, which then results in an increase in endogenous cytokinin. This leads to a feedback response involving an upregulation of *CKX* expression and/or an increase in the activity of the CKX enzyme. The gene expression data implicates *BrCKX3* as a strong candidate for this role. The model also indicates that *IPT* expression could be downregulated soon after INCYDE treatment, and then subsequently increased as cytokinin levels decrease. The gene expression data indicate that *BrIPT1* and/or *BrIPT3* could fulfil this role. The model of the feedback response to INCYDE (**Figure 2.4**) has been updated to reflect the input of the candidate genes (**Figure 6.1**). In wheat leaves, the sustained upregulation of *TaCKX8* and downregulation of *TaIPT2* following INCYDE treatment (**Figure 5.7B**) relative to the control (**Figures 5.7A** and **5.7C**), suggests a potential feedback response in this monocot that also normalises endogenous cytokinin levels.

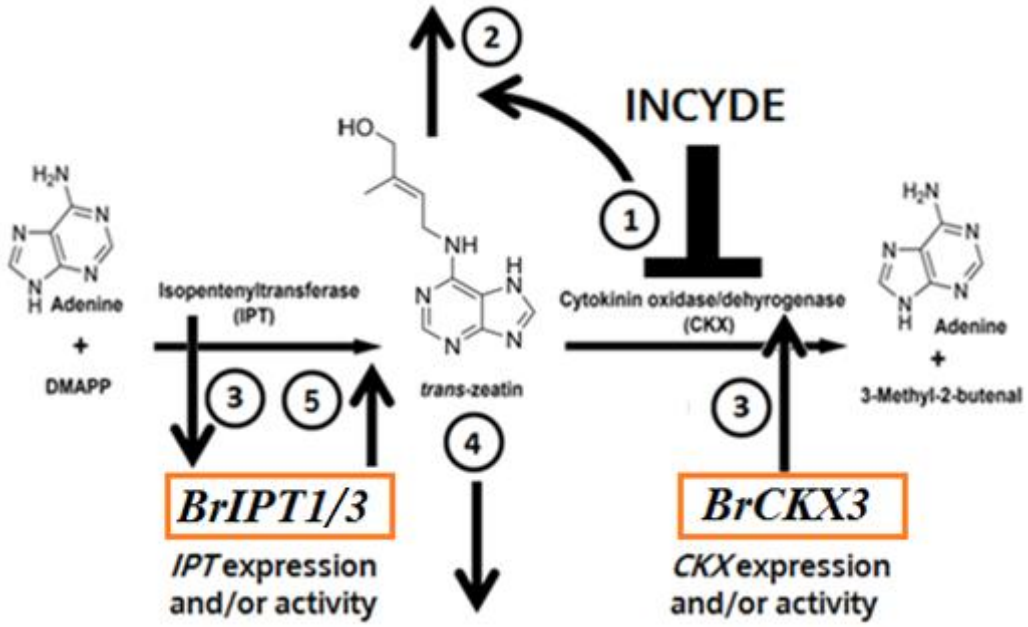

Figure 6.1 The feedback model for the cytokinin response to INCYDE treatment. 1: INCYDE inhibits CKX; 2: This leads to an increase in cytokinin (such as *t*Z); 3: a feedback response is activated involving upregulating *CKX* (such as *BrCKX3* in rapid cycling *B. rapa*) and downregulating *IPT* (such as *BrIPT1* and/or *BrIPT3*). 4: The concentration of cytokinin reduces and; 5: results in a corrective increase in *IPT* (*BrIPT1*).

TDZ-K was confirmed to not inhibit root growth (**Table 2.6**), as purported (United States Patent US 2017/0280721 A1, 2017). TDZ-K application had a strong effect on the upregulation of *BrIPT*s and *BrCKX*s relative to the controls, and the expression showed some parallels with the response to INCYDE, with a strong increase in *BrCKX3* expression one day after treatment (**Figure 5.13**). This combined with the previously-described evidence of TDZ-K affecting the gene expression of *TaRRA4*s, which itself is a good indicator of changes in endogenous cytokinin, confirms that TDZ-K affects cytokinin homeostasis.

Gene expression analyses of CPPU-treated RCBr, showed little effect on *BrIPT* or *BrCKX* (**Figure 5.16**), suggesting that future analyses should focus on other stages of development, other than mid-flowering. Ultimately, the differential effects of each PGR on the expression of genes associated with cytokinin provided evidence for different biological mechanisms of each and the sensitivity of cytokinin regulatory genes to these exogenously applied compounds.

6.3 Future approaches and analyses

It is evident that more work is required to fully understand the effects of INCYDE, TDZ-K and CPPU on the growth, yield and development of cereals and RCBr, and to determine the mechanism of action of each and the effect of each compound on cytokinin homeostasis. Future field work with cereals should focus on applying the PGRs at earlier growth stages, at the end of elongation (GS 39) and earlier with INCYDE and CPPU, with GS 39 being a promising stage where enhancements in yield have been observed, and where there is a growing body of genetic evidence to suggest targeting the developing inflorescence at this stage (Yamburenko *et al*., 2017). TDZ-K should be targeted at head emergence and at earlier growth stages, on the basis of yield enhancements observed recently (United States Patent US 2017/0280721 A1, 2017). Further work with RCBr should determine the effect of different surfactants on the efficacy of each PGR, and how treatments and application can be optimised, to more closely replicate the growing conditions used in experiments at Palacký University.

Given the difficulties and complexities of accounting for the contributing effect of the environment and various stress conditions (Zwack and Rashotte, 2015), it might be more suitable for future cereal experiments to be carried out under more controlled conditions. For example, drought experiments should be conducted in a controlled growth environment using polyethylene glycol (PEG) (Rauf *et al*., 2007). Future research should also determine the biological significance of the observed increase in conjugated cytokinin forms following INCYDE treatment, and should extend LC-MS/MS analyses to cover more time points to determine the effect of INCYDE on wheat grains, which was missed due to the *Septoria* issue in the first trial. Analysing more time points will provide detailed insight into cytokinin homeostasis during a period of development in which cytokinin levels are rapidly changing. Additionally, future analyses of endogenous cytokinin in grains should determine the cytokinin content in specific parts within the grain and in different subcellular locations, since significant differences in the concentration of different cytokinin types at a subcellular level might have been missed. The vacuole is one subcellular location that should be focused on. The increased *O*-glucoside type cytokinins in barley grain following INCYDE treatment (**Table 5.4**) are likely to accumulate in vacuoles (Jiskrová *et al*., 2016; Pospíšilová *et al*., 2016). Additionally, *CKX* expression and/or activity, which is inhibited by INCYDE, is localised around the vacuole (Werner *et al*., 2003; Kowalska *et al*., 2010), indicating that this part of the cell might provide more insight into the biological mechanism of INCYDE and CPPU.

Future experiments should expand on the complement of genes studied, to include more cell wall invertases (*CWINV*), as well as sucrose transporters (*SUT*) and cytokinin-activating LONELY GUY (*LOG*). Expression studies should also focus on earlier time frames before 24 h following treatments, to ensure rapid and transient responses are not missed. Gene expression analyses should be carried out on the other parts of RCBr, including the meristem tissue, which has a fundamental role in influencing flowering and determining yield (Ashikari *et al*., 2005; Bartrina *et al*., 2011; Jameson and Song, 2016). The gene expression analyses highlighted some key *IPT* and *CKX* gene family members in wheat and RCBr and their response to PGRs, and future gene expression work should focus on the specific role of each of these. Rapid cycling *B. rapa* has been shown to be an effective model plant for investigating the effect of PGRs, and could be used in future analyses.

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Appendices

Appendix 1: Recipes and protocols

1.1 Gel electrophoresis

1.1.1 Agarose gel (1%)

Mix 0.3 g agarose (HydraGene) with 30 mL 1 x TAE buffer
Add 2 µL of SYBR™ Safe DNA Gel Stain (Thermo Fisher Scientific)

Pour the gel after cooling

Allow to solidify after 30 min, load the HyperLadderTM 1 kb (Bioline)

Add 2 µL sample, 3 µL TAE and 1 µL 6x agarose gel loading dye (6 µL total) to each well

1.1.2 6x agarose gel loading dye

60% Glycerol

60 mM EDTA

10 mM Tris-HCl (pH 7.6)

0.03% xylene cyanol FF

0.03% bromophenol blue

1.1.3 25 x TAE buffer

121 g Tris base

28.5 mL Glacial acetic acid

9.3 g EDTA

Adjust pH = 7.6 and adjust volume to 1 L with distilled H_2O

1.2 cDNA synthesis

1.2.1 Primer annealing mix

2-7.5 µL (0.5-1 µg total) RNA

1 µL (1 µg) 100 pmoles (0.2 µg) Random pd(N)6 (Sigma-Aldrich)

1 µL (1 µg) 50 pmoles/0.25 µg Oligo(dT)18 primers (Bioline)

0.5 µL 25x of RNA secure™ (Thermo Fisher Scientific)

DEPC-treated water added to final volume of 10 µL

Heat to 65 ºC for 10 min and transfer to ice immediately for 2 minutes. Spin the primer-RNA mix.

1.2.2 Reverse transcriptase mix

To the primer annealing mix (**Appendix 1.2.1**) add 10 µL of the following:

4 µL 5x RT buffer (Sigma-Aldrich)

1 µL 20 mM dNTPs (Thermo Fisher Scientific)

2 µL DTT (Sigma-Aldrich)

2 µL DEPC-treated water

1 µL Expand™ Reverse Transcriptase (Sigma-Aldrich)

1.3 SYBR Green solution

1.3.1 2x SYBR Green

For 1 mL:

930 µL qPCR buffer

40 µL 20 mM dNTP (Thermo Fisher Scientific)

15 µL 100x SYBR™ Green I Nucleic Acid Gel Stain (Thermo Fisher Scientific)

15 µL BIOTAQ™ DNA Polymerase (Bioline)

1.3.2 qPCR buffer recipe

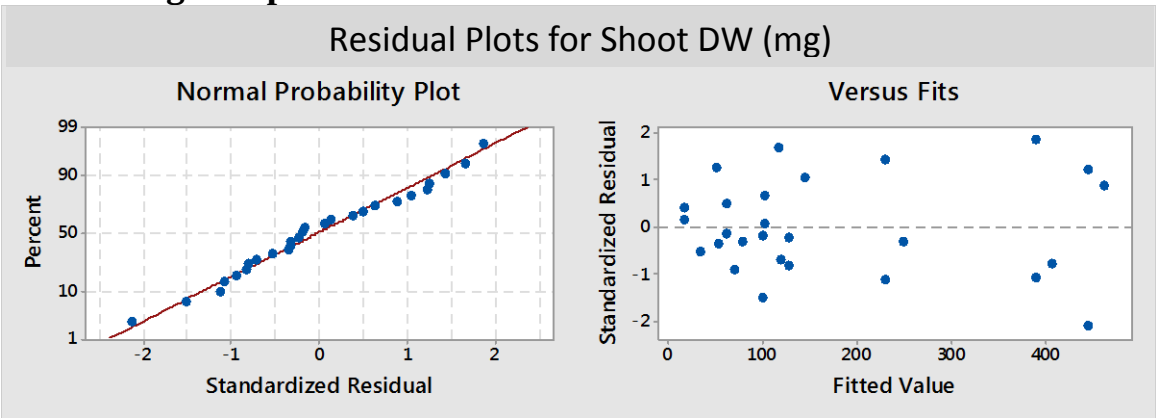
For 1 mL:

227 mg	Trehalose (Lab Supply)
340 µL	DEPC-treated water
200 µL	10x PCR buffer (-Mg) (Bioline)
160 µL	50 mM $MgCl_2$ (Bioline)
80 µL	DMSO
30 µL	10% Triton-X 100

Appendix 2: Chapter 2 statistical analyses for growth room experiments with rapid cycling *Brassica rapa*

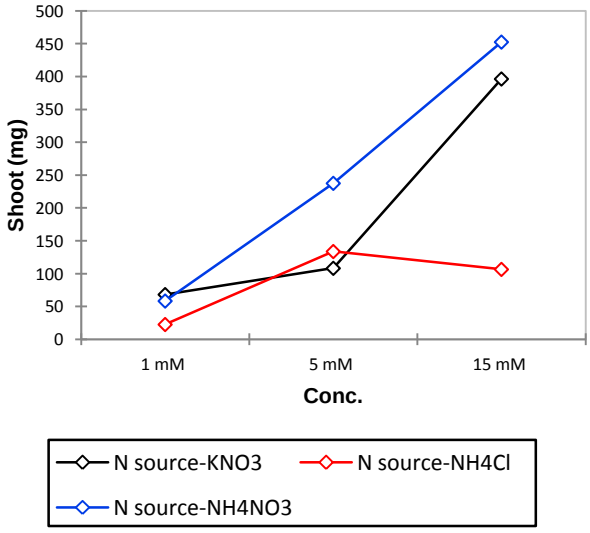
2.1 Nitrogen experiment

2.1.1 Nitrogen experiment: Shoot DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	1906	953	0.67	0.528
Conc.	2	328629	164314	114.67	0.000
N source	2	120885	60442	42.18	0.000
Conc.*N source	4	117658	29415	20.53	0.000
Error	16	22926	1433		
Total	26	592004			



Conc. / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Shoot (mg)):								
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant			
15 mM vs 1 mM	268.778	15.062	2.580	< **0.0001**	Yes			
15 mM vs 5 mM	158.685	8.893	2.580	< **0.0001**	Yes			
5 mM vs 1 mM	110.093	6.170	2.580	< **0.0001**	Yes			
Tukey's d critical value:			3.649					
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
15 mM	318.704	12.618	291.955	345.452	A			
5 mM	160.019	12.618	133.270	186.767		B		
1 mM	49.926	12.618	23.177	76.675			C	
N source / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Shoot (mg)):								
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant			
NH4NO3 vs NH4Cl	161.852	9.070	2.580	< **0.0001**	Yes			
NH4NO3 vs KNO3	58.556	3.281	2.580	**0.012**	Yes			
KNO3 vs NH4Cl	103.296	5.789	2.580	< **0.0001**	Yes			
Tukey's d critical value:			3.649					
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
NH4NO3	249.685	12.618	222.936	276.434	A			
KNO3	191.130	12.618	164.381	217.878		B		
NH4Cl	87.833	12.618	61.085	114.582			C	
Conc.*N source / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Shoot (mg)):								
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant			
Conc.-15 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4Cl	429.944	13.911	3.557	< **0.0001**	Yes			
Conc.-15 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4NO3	394.222	12.755	3.557	< **0.0001**	Yes			
Conc.-15 mM *N source-NH4NO3 vs Conc.-1 mM *N source-KNO3	384.389	12.437	3.557	< **0.0001**	Yes			
Conc.-15 mM *N source-NH4NO3 vs Conc.-15 mM *N source-NH4Cl	346.056	11.197	3.557	< **0.0001**	Yes			
Conc.-15 mM *N source-NH4NO3 vs Conc.-5 mM *N source-KNO3	344.389	11.143	3.557	< **0.0001**	Yes			
Conc.-15 mM *N source-NH4NO3 vs Conc.-5 mM *N	318.833	10.316	3.557	< **0.0001**	Yes			

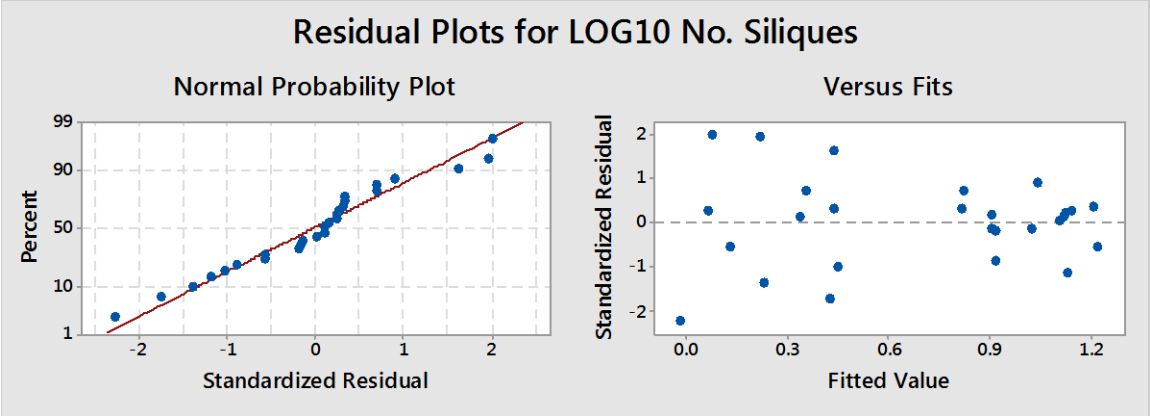
source-NH4Cl								
Conc.-15 mM *N source-NH4NO3 vs Conc.-5 mM *N source-NH4NO3	215.056	6.958	3.557	< **0.0001**	Yes			
Conc.-15 mM *N source-NH4NO3 vs Conc.-15 mM *N source-KNO3	56.167	1.817	3.557	0.672	No			
Conc.-15 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4Cl	373.778	12.093	3.557	< **0.0001**	Yes			
Conc.-15 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4NO3	338.056	10.938	3.557	< **0.0001**	Yes			
Conc.-15 mM *N source-KNO3 vs Conc.-1 mM *N source-KNO3	328.222	10.620	3.557	< **0.0001**	Yes			
Conc.-15 mM *N source-KNO3 vs Conc.-15 mM *N source-NH4Cl	289.889	9.379	3.557	< **0.0001**	Yes			
Conc.-15 mM *N source-KNO3 vs Conc.-5 mM *N source-KNO3	288.222	9.325	3.557	< **0.0001**	Yes			
Conc.-15 mM *N source-KNO3 vs Conc.-5 mM *N source-NH4Cl	262.667	8.498	3.557	< **0.0001**	Yes			
Conc.-15 mM *N source-KNO3 vs Conc.-5 mM *N source-NH4NO3	158.889	5.141	3.557	**0.002**	Yes			
Conc.-5 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4Cl	214.889	6.953	3.557	< **0.0001**	Yes			
Conc.-5 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4NO3	179.167	5.797	3.557	**0.001**	Yes			
Conc.-5 mM *N source-NH4NO3 vs Conc.-1 mM *N source-KNO3	169.333	5.479	3.557	**0.001**	Yes			
Conc.-5 mM *N source-NH4NO3 vs Conc.-15 mM *N source-NH4Cl	131.000	4.238	3.557	**0.014**	Yes			
Conc.-5 mM *N source-NH4NO3 vs Conc.-5 mM *N source-KNO3	129.333	4.185	3.557	**0.015**	Yes			
Conc.-5 mM *N source-NH4NO3 vs Conc.-5 mM *N source-NH4Cl	103.778	3.358	3.557	0.072	No			
Conc.-5 mM *N source-NH4Cl vs Conc.-1 mM *N source-NH4Cl	111.111	3.595	3.557	**0.047**	Yes			
Conc.-5 mM *N source-NH4Cl vs Conc.-1 mM *N source-NH4NO3	75.389	2.439	3.557	0.327	No			
Conc.-5 mM *N source-NH4Cl vs Conc.-1 mM *N source-KNO3	65.556	2.121	3.557	0.493	No			
Conc.-5 mM *N source-NH4Cl vs Conc.-15 mM *N source-NH4Cl	27.222	0.881	3.557	0.991	No			
Conc.-5 mM *N source-NH4Cl vs Conc.-5 mM *N source-KNO3	25.556	0.827	3.557	0.994	No			

Conc.-5 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4Cl	85.556	2.768	3.557	0.199	No			
Conc.-5 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4NO3	49.833	1.612	3.557	0.786	No			
Conc.-5 mM *N source-KNO3 vs Conc.-1 mM *N source-KNO3	40.000	1.294	3.557	0.919	No			
Conc.-5 mM *N source-KNO3 vs Conc.-15 mM *N source-NH4Cl	1.667	0.054	3.557	1.000	No			
Conc.-15 mM *N source-NH4Cl vs Conc.-1 mM *N source-NH4Cl	83.889	2.714	3.557	0.217	No			
Conc.-15 mM *N source-NH4Cl vs Conc.-1 mM *N source-NH4NO3	48.167	1.558	3.557	0.813	No			
Conc.-15 mM *N source-NH4Cl vs Conc.-1 mM *N source-KNO3	38.333	1.240	3.557	0.935	No			
Conc.-1 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4Cl	45.556	1.474	3.557	0.852	No			
Conc.-1 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4NO3	9.833	0.318	3.557	1.000	No			
Conc.-1 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4Cl	35.722	1.156	3.557	0.955	No			
Tukey's d critical value:			5.031					
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
Conc.-15 mM *N source-NH4NO3	452.778	21.855	406.448	499.108	A			
Conc.-15 mM *N source-KNO3	396.611	21.855	350.281	442.941	A			
Conc.-5 mM *N source-NH4NO3	237.722	21.855	191.392	284.052		B		
Conc.-5 mM *N source-NH4Cl	133.944	21.855	87.614	180.275		B	C	
Conc.-5 mM *N source-KNO3	108.389	21.855	62.059	154.719			C	D
Conc.-15 mM *N source-NH4Cl	106.722	21.855	60.392	153.052			C	D
Conc.-1 mM *N source-KNO3	68.389	21.855	22.059	114.719			C	D
Conc.-1 mM *N source-NH4NO3	58.556	21.855	12.225	104.886			C	D
Conc.-1 mM *N source-NH4Cl	22.833	21.855	-23.497	69.164				D

Summary of all pairwise comparisons for Conc. (Tukey (HSD)):					
Category	LS means(Shoot (mg))	Groups			
15 mM	318.704	A			
5 mM	160.019		B		
1 mM	49.926			C	
Summary of all pairwise comparisons for N source (Tukey (HSD)):					
Category	LS means(Shoot (mg))	Groups			
NH4NO3	249.685	A			
KNO3	191.130		B		

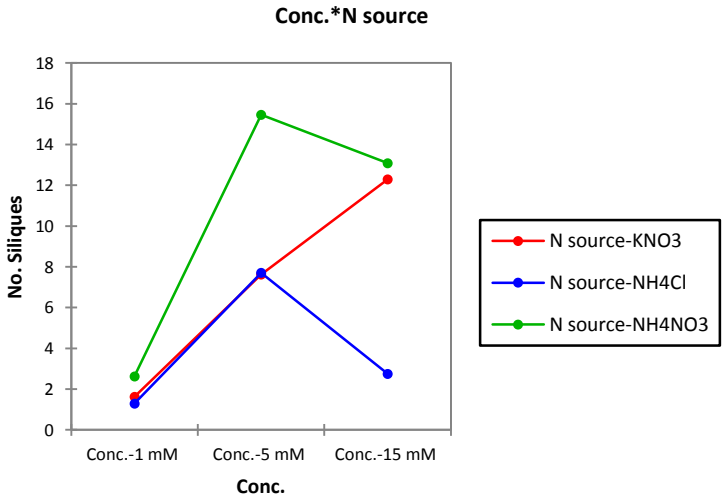
NH4Cl	87.833			C	
Summary of all pairwise comparisons for Conc.*N source (Tukey (HSD)):					
Category	LS means(Shoot (mg))	Groups			
15 mM *NH4NO3	452.778	A			
15 mM *KNO3	396.611	A			
5 mM *NH4NO3	237.722		B		
5 mM *NH4Cl	133.944		B	C	
5 mM *KNO3	108.389			C	D
15 mM *NH4Cl	106.722			C	D
1 mM *KNO3	68.389			C	D
1 mM *NH4NO3	58.556			C	D
1 mM *NH4Cl	22.833				D

2.1.2 Nitrogen experiment: Silique number



Analysis of Variance

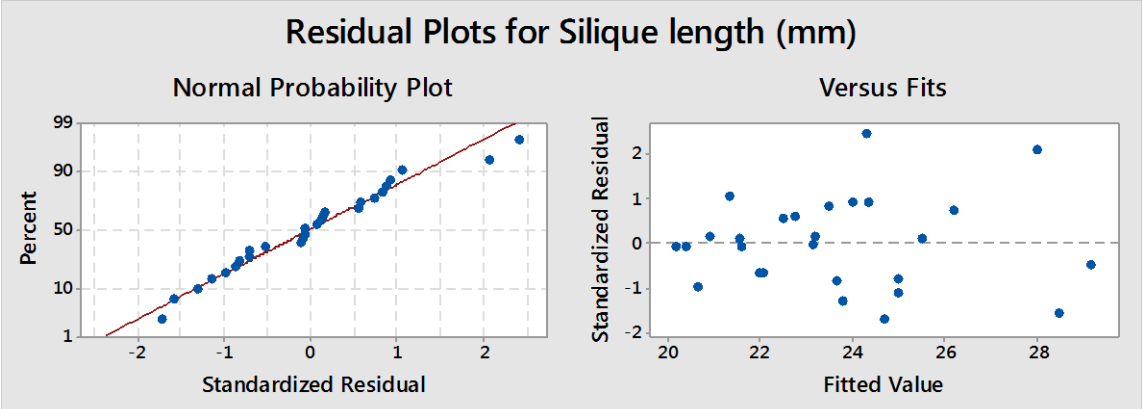
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.05310	0.02655	1.03	0.378
Conc.	2	3.07994	1.53997	59.95	**0.000**
N source	2	0.97036	0.48518	18.89	**0.000**
Conc.*N source	4	0.39551	0.09888	3.85	**0.022**
Error	16	0.41102	0.02569		
Total	26	4.90994			



Conc. / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (LOG10 No. Siliques):						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
5 mM vs 1 mM	0.768	10.171	2.580	**< 0.0001**	Yes	
5 mM vs 15 mM	0.119	1.573	2.580	0.285	No	
15 mM vs 1 mM	0.650	8.598	2.580	**< 0.0001**	Yes	
Tukey's d critical value:			3.649			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
5 mM	0.984	0.053	0.871	1.097	A	
15 mM	0.865	0.053	0.752	0.979	A	
1 mM	0.216	0.053	0.102	0.329		B
N source / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (LOG10 No. Siliques):						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
NH4NO3 vs NH4Cl	0.461	6.104	2.580	**< 0.0001**	Yes	
NH4NO3 vs KNO3	0.184	2.429	2.580	0.067	No	
KNO3 vs NH4Cl	0.278	3.675	2.580	**0.005**	Yes	
Tukey's d critical value:			3.649			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
NH4NO3	0.903	0.053	0.790	1.017	A	
KNO3	0.720	0.053	0.607	0.833	A	
NH4Cl	0.442	0.053	0.329	0.555		B
Conc.*N source / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (LOG10 No. Siliques):						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
Conc.-5 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4Cl	1.146	8.754	3.557	**< 0.0001**	Yes	
Conc.-5 mM *N source-NH4NO3 vs Conc.-1 mM *N source-KNO3	0.995	7.604	3.557	**< 0.0001**	Yes	
Conc.-5 mM *N source-NH4NO3 vs Conc.-15 mM *N source-NH4Cl	0.785	6.001	3.557	**0.000**	Yes	
Conc.-5 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4NO3	0.772	5.899	3.557	**0.001**	Yes	
Conc.-5 mM *N source-NH4NO3 vs Conc.-5 mM *N source-KNO3	0.305	2.328	3.557	0.381	No	
Conc.-5 mM *N source-NH4NO3 vs Conc.-5 mM *N source-NH4Cl	0.303	2.312	3.557	0.389	No	
Conc.-5 mM *N source-NH4NO3 vs Conc.-15 mM *N source-KNO3	0.101	0.770	3.557	0.996	No	
Conc.-5 mM *N source-NH4NO3 vs Conc.-15 mM *N source-NH4NO3	0.078	0.595	3.557	0.999	No	
Conc.-15 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4Cl	1.068	8.159	3.557	**< 0.0001**	Yes	

Conc.-15 mM *N source-NH4NO3 vs Conc.-1 mM *N source-KNO3	0.917	7.009	3.557	< **0.0001**	Yes	
Conc.-15 mM *N source-NH4NO3 vs Conc.-15 mM *N source-NH4Cl	0.707	5.406	3.557	**0.001**	Yes	
Conc.-15 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4NO3	0.694	5.304	3.557	**0.002**	Yes	
Conc.-15 mM *N source-NH4NO3 vs Conc.-5 mM *N source-KNO3	0.227	1.733	3.557	0.720	No	
Conc.-15 mM *N source-NH4NO3 vs Conc.-5 mM *N source-NH4Cl	0.225	1.717	3.557	0.729	No	
Conc.-15 mM *N source-NH4NO3 vs Conc.-15 mM *N source-KNO3	0.023	0.175	3.557	1.000	No	
Conc.-15 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4Cl	1.045	7.984	3.557	< **0.0001**	Yes	
Conc.-15 mM *N source-KNO3 vs Conc.-1 mM *N source-KNO3	0.894	6.834	3.557	**0.000**	Yes	
Conc.-15 mM *N source-KNO3 vs Conc.-15 mM *N source-NH4Cl	0.685	5.231	3.557	**0.002**	Yes	
Conc.-15 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4NO3	0.671	5.130	3.557	**0.002**	Yes	
Conc.-15 mM *N source-KNO3 vs Conc.-5 mM *N source-KNO3	0.204	1.558	3.557	0.813	No	
Conc.-15 mM *N source-KNO3 vs Conc.-5 mM *N source-NH4Cl	0.202	1.542	3.557	0.821	No	
Conc.-5 mM *N source-NH4Cl vs Conc.-1 mM *N source-NH4Cl	0.843	6.442	3.557	**0.000**	Yes	
Conc.-5 mM *N source-NH4Cl vs Conc.-1 mM *N source-KNO3	0.693	5.292	3.557	**0.002**	Yes	
Conc.-5 mM *N source-NH4Cl vs Conc.-15 mM *N source-NH4Cl	0.483	3.689	3.557	**0.039**	Yes	
Conc.-5 mM *N source-NH4Cl vs Conc.-1 mM *N source-NH4NO3	0.469	3.587	3.557	**0.047**	Yes	
Conc.-5 mM *N source-NH4Cl vs Conc.-5 mM *N source-KNO3	0.002	0.016	3.557	1.000	No	
Conc.-5 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4Cl	0.841	6.426	3.557	**0.000**	Yes	
Conc.-5 mM *N source-KNO3 vs Conc.-1 mM *N source-KNO3	0.690	5.276	3.557	**0.002**	Yes	
Conc.-5 mM *N source-KNO3 vs Conc.-15 mM *N source-NH4Cl	0.481	3.673	3.557	**0.040**	Yes	
Conc.-5 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4NO3	0.467	3.571	3.557	**0.049**	Yes	
Conc.-1 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4Cl	0.374	2.854	3.557	0.173	No	
Conc.-1 mM *N source-NH4NO3 vs Conc.-1 mM *N source-KNO3	0.223	1.705	3.557	0.736	No	
Conc.-1 mM *N source-NH4NO3 vs Conc.-15 mM *N source-NH4Cl	0.013	0.101	3.557	1.000	No	
Conc.-15 mM *N source-NH4Cl vs Conc.-1 mM *N source-NH4Cl	0.360	2.753	3.557	0.204	No	
Conc.-15 mM *N source-NH4Cl vs Conc.-1 mM *N source-KNO3	0.210	1.603	3.557	0.790	No	
Conc.-1 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4Cl	0.150	1.150	3.557	0.956	No	
Tukey's d critical value:			5.031			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
Conc.-5 mM *N source-NH4NO3	1.187	0.093	0.990	1.383	A	
Conc.-15 mM *N source-NH4NO3	1.109	0.093	0.913	1.305	A	
Conc.-15 mM *N source-KNO3	1.086	0.093	0.890	1.282	A	
Conc.-5 mM *N source-NH4Cl	0.884	0.093	0.688	1.080	A	
Conc.-5 mM *N source-KNO3	0.882	0.093	0.686	1.078	A	
Conc.-1 mM *N source-NH4NO3	0.415	0.093	0.218	0.611		B
Conc.-15 mM *N source-NH4Cl	0.401	0.093	0.205	0.598		B
Conc.-1 mM *N source-KNO3	0.192	0.093	-0.005	0.388		B
Conc.-1 mM *N source-NH4Cl	0.041	0.093	-0.155	0.237		B

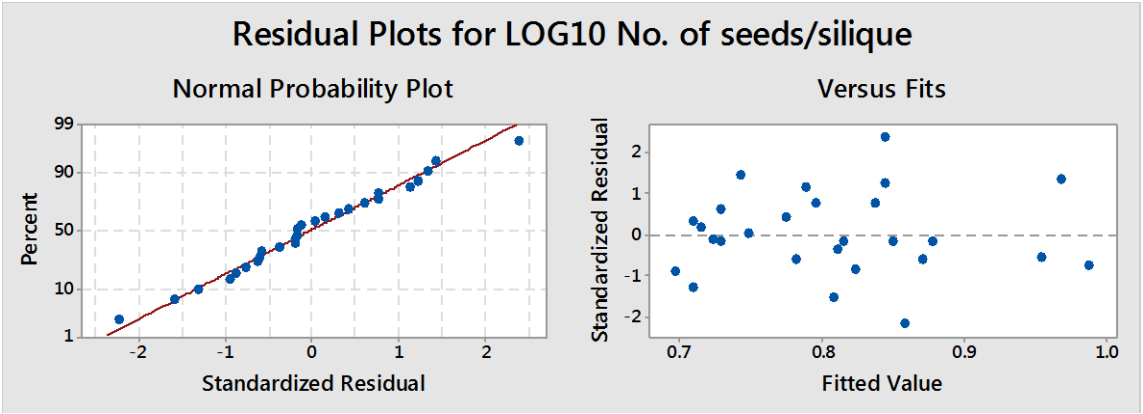
2.1.3 Nitrogen experiment: Silique length



Analysis of Variance

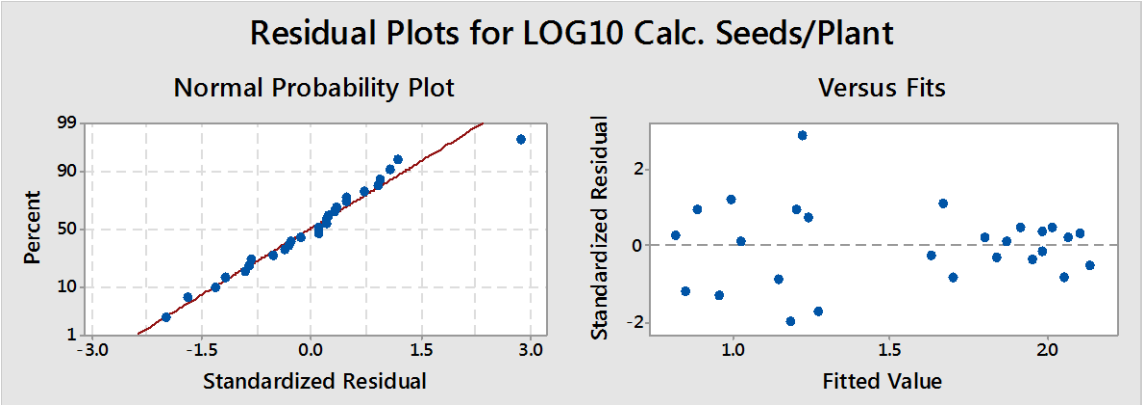
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	6.387	3.193	0.39	0.680
Conc.	2	28.548	14.274	1.76	0.203
N source	2	30.965	15.483	1.91	0.180
Conc.*N source	4	83.462	20.865	2.58	0.077
Error	16	129.555	8.097		
Total	26	278.917			

2.1.4 Nitrogen experiment: Seeds per silique

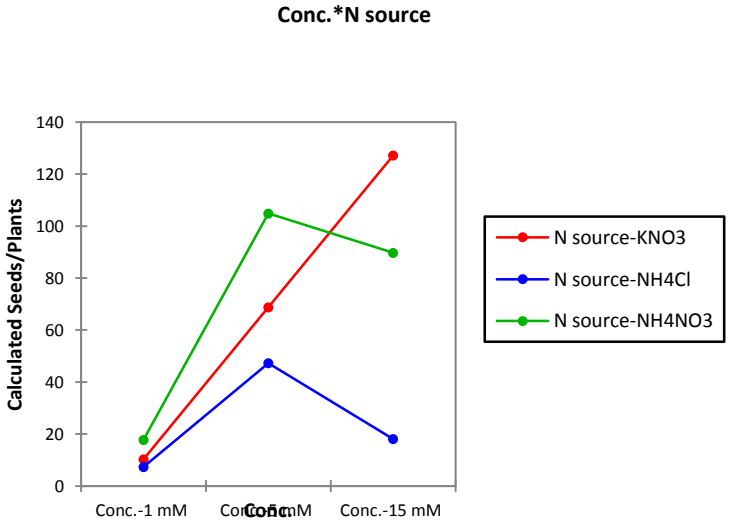


Analysis of Variance					
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.004971	0.002486	0.21	0.810
Conc.	2	0.037778	0.018889	1.62	0.228
N source	2	0.026308	0.013154	1.13	0.347
Conc.*N source	4	0.097134	0.024283	2.09	0.130
Error	16	0.186061	0.011629		
Total	26	0.352252			

2.1.5 Nitrogen experiment: Seeds per plant



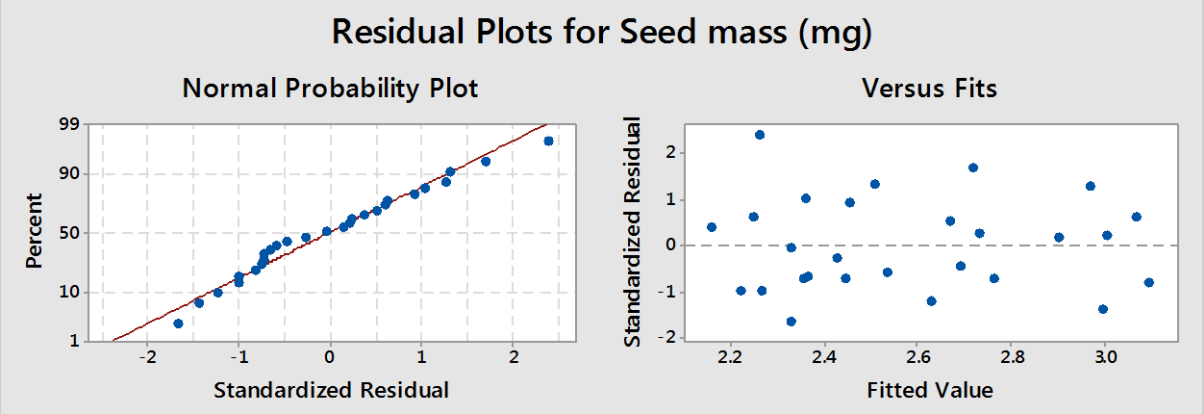
Analysis of Variance					
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.02271	0.01136	0.61	0.554
Conc.	2	3.55002	1.77501	95.81	0.000
N source	2	1.28088	0.64044	34.57	0.000
Conc.*N source	4	0.58427	0.14607	7.88	0.001
Error	16	0.29641	0.01853		
Total	26	5.73429			



Conc. / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (LOG10 Calc. Seeds/Plant):							
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant		
5 mM vs 1 mM	0.812	12.648	2.580	< **0.0001**	Yes		
5 mM vs 15 mM	0.093	1.451	2.580	0.340	No		
15 mM vs 1 mM	0.718	11.197	2.580	< **0.0001**	Yes		
Tukey's d critical value:			3.649				
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
5 mM	1.841	0.045	1.744	1.937	A		
15 mM	1.747	0.045	1.651	1.844	A		
1 mM	1.029	0.045	0.933	1.125		B	
N source / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (LOG10 Calc. Seeds/Plant):							
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant		
NH4NO3 vs NH4Cl	0.501	7.807	2.580	< **0.0001**	Yes		
NH4NO3 vs KNO3	0.091	1.426	2.580	0.352	No		
KNO3 vs NH4Cl	0.409	6.382	2.580	< **0.0001**	Yes		
Tukey's d critical value:			3.649				
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
NH4NO3	1.736	0.045	1.640	1.833	A		
KNO3	1.645	0.045	1.549	1.741	A		
NH4Cl	1.235	0.045	1.139	1.332		B	
Conc.*N source / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (LOG10 Calc. Seeds/Plant):							
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant		
Conc.-15 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4Cl	1.250	11.252	3.557	< **0.0001**	Yes		
Conc.-15 mM *N source-KNO3 vs Conc.-1 mM *N source-KNO3	1.109	9.978	3.557	< **0.0001**	Yes		
Conc.-15 mM *N source-KNO3 vs Conc.-15 mM *N source-NH4Cl	0.918	8.257	3.557	< **0.0001**	Yes		
Conc.-15 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4NO3	0.865	7.785	3.557	< **0.0001**	Yes		
Conc.-15 mM *N source-KNO3 vs Conc.-5 mM *N source-NH4Cl	0.437	3.931	3.557	**0.025**	Yes		
Conc.-15 mM *N source-KNO3 vs Conc.-5 mM *N source-KNO3	0.268	2.409	3.557	0.342	No		
Conc.-15 mM *N source-KNO3 vs Conc.-15 mM *N source-NH4NO3	0.152	1.364	3.557	0.896	No		
Conc.-15 mM *N source-KNO3 vs Conc.-5 mM *N source-NH4NO3	0.085	0.768	3.557	0.996	No		
Conc.-5 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4Cl	1.165	10.484	3.557	< **0.0001**	Yes		
Conc.-5 mM *N source-NH4NO3 vs Conc.-1 mM *N source-KNO3	1.023	9.210	3.557	< **0.0001**	Yes		
Conc.-5 mM *N source-NH4NO3 vs Conc.-15 mM *N source-NH4Cl	0.832	7.489	3.557	< **0.0001**	Yes		
Conc.-5 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4NO3	0.780	7.017	3.557	< **0.0001**	Yes		
Conc.-5 mM *N source-NH4NO3 vs Conc.-5 mM *N source-NH4Cl	0.351	3.163	3.557	0.102	No		
Conc.-5 mM *N source-NH4NO3 vs Conc.-5 mM *N source-KNO3	0.182	1.640	3.557	0.771	No		

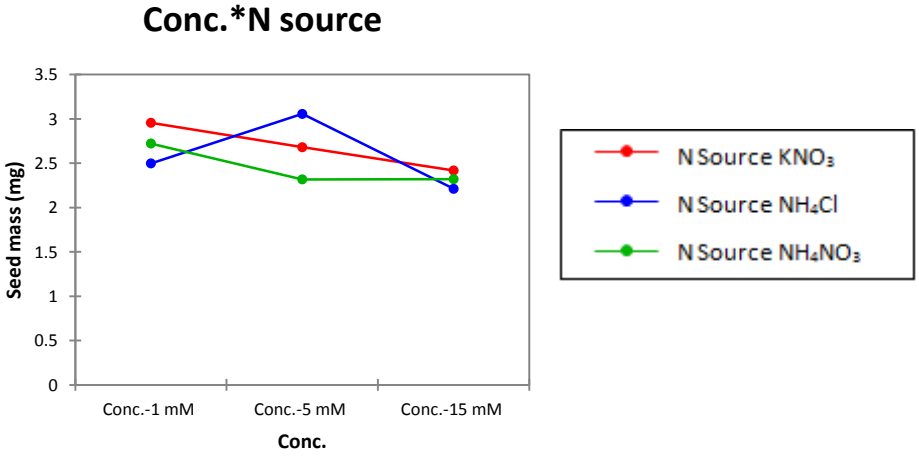
Conc.-5 mM *N source-NH4NO3 vs Conc.-15 mM *N source-NH4NO3	0.066	0.596	3.557	0.999	No		
Conc.-15 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4Cl	1.099	9.888	3.557	< **0.0001**	Yes		
Conc.-15 mM *N source-NH4NO3 vs Conc.-1 mM *N source-KNO3	0.957	8.614	3.557	< **0.0001**	Yes		
Conc.-15 mM *N source-NH4NO3 vs Conc.-15 mM *N source-NH4Cl	0.766	6.893	3.557	< **0.0001**	Yes		
Conc.-15 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4NO3	0.714	6.421	3.557	**0.000**	Yes		
Conc.-15 mM *N source-NH4NO3 vs Conc.-5 mM *N source-NH4Cl	0.285	2.567	3.557	0.272	No		
Conc.-15 mM *N source-NH4NO3 vs Conc.-5 mM *N source-KNO3	0.116	1.044	3.557	0.975	No		
Conc.-5 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4Cl	0.983	8.843	3.557	< **0.0001**	Yes		
Conc.-5 mM *N source-KNO3 vs Conc.-1 mM *N source-KNO3	0.841	7.569	3.557	< **0.0001**	Yes		
Conc.-5 mM *N source-KNO3 vs Conc.-15 mM *N source-NH4Cl	0.650	5.849	3.557	**0.001**	Yes		
Conc.-5 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4NO3	0.597	5.376	3.557	**0.002**	Yes		
Conc.-5 mM *N source-KNO3 vs Conc.-5 mM *N source-NH4Cl	0.169	1.522	3.557	0.830	No		
Conc.-5 mM *N source-NH4Cl vs Conc.-1 mM *N source-NH4Cl	0.814	7.321	3.557	< **0.0001**	Yes		
Conc.-5 mM *N source-NH4Cl vs Conc.-1 mM *N source-KNO3	0.672	6.047	3.557	**0.000**	Yes		
Conc.-5 mM *N source-NH4Cl vs Conc.-15 mM *N source-NH4Cl	0.481	4.327	3.557	**0.012**	Yes		
Conc.-5 mM *N source-NH4Cl vs Conc.-1 mM *N source-NH4NO3	0.428	3.854	3.557	**0.029**	Yes		
Conc.-1 mM *N source-NH4NO3 vs Conc.-1 mM *N source-NH4Cl	0.385	3.467	3.557	0.059	No		
Conc.-1 mM *N source-NH4NO3 vs Conc.-1 mM *N source-KNO3	0.244	2.193	3.557	0.453	No		
Conc.-1 mM *N source-NH4NO3 vs Conc.-15 mM *N source-NH4Cl	0.053	0.472	3.557	1.000	No		
Conc.-15 mM *N source-NH4Cl vs Conc.-1 mM *N source-NH4Cl	0.333	2.995	3.557	0.137	No		
Conc.-15 mM *N source-NH4Cl vs Conc.-1 mM *N source-KNO3	0.191	1.721	3.557	0.727	No		
Conc.-1 mM *N source-KNO3 vs Conc.-1 mM *N source-NH4Cl	0.142	1.274	3.557	0.925	No		
Tukey's d critical value:			5.031				
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Conc.-15 mM *N source-KNO3	2.104	0.079	1.937	2.270	A		
Conc.-5 mM *N source-NH4NO3	2.018	0.079	1.852	2.185	A	B	
Conc.-15 mM *N source-NH4NO3	1.952	0.079	1.786	2.119	A	B	
Conc.-5 mM *N source-KNO3	1.836	0.079	1.670	2.003	A	B	
Conc.-5 mM *N source-NH4Cl	1.667	0.079	1.500	1.834		B	
Conc.-1 mM *N source-NH4NO3	1.239	0.079	1.072	1.405			C
Conc.-15 mM *N source-NH4Cl	1.186	0.079	1.020	1.353			C
Conc.-1 mM *N source-KNO3	0.995	0.079	0.828	1.162			C
Conc.-1 mM *N source-NH4Cl	0.853	0.079	0.687	1.020			C

2.1.6 Nitrogen experiment: Seed mass



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.03971	0.01985	0.99	0.394
Conc.	2	0.91315	0.45657	22.75	**0.000**
N source	2	0.24258	0.12129	6.04	**0.011**
Conc.*N source	4	0.95249	0.23812	11.86	**0.000**
Error	16	0.32112	0.02007		
Total	26	2.46905			

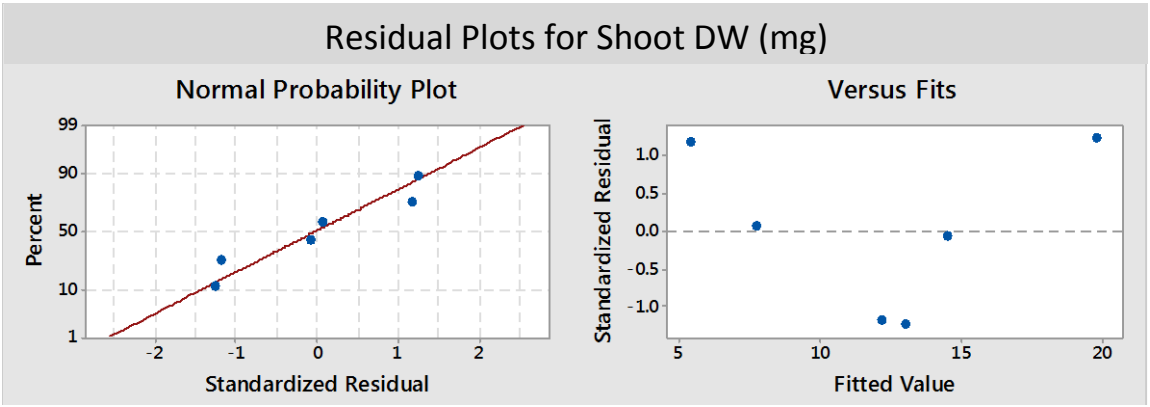


Conc. / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:							
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant		
1 mM vs 15 mM	0.409	6.123	2.580	< 0.0001	Yes		
1 mM vs 5 mM	0.041	0.610	2.580	0.817	No		
5 mM vs 15 mM	0.368	5.513	2.580	0.000	Yes		
Tukey's d critical value:			3.649				
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
1 mM	2.725	0.047	2.625	2.825	A		
5 mM	2.684	0.047	2.584	2.785	A		
15 mM	2.316	0.047	2.216	2.416		B	
N source / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:							
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant		
KNO3 vs NH4NO3	0.231	3.461	2.580	0.009	Yes		
KNO3 vs NH4Cl	0.096	1.442	2.580	0.344	No		
NH4Cl vs NH4NO3	0.135	2.019	2.580	0.140	No		
Tukey's d critical value:			3.649				
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
KNO3	2.684	0.047	2.584	2.785	A		
NH4Cl	2.588	0.047	2.488	2.688	A	B	
NH4NO3	2.453	0.047	2.353	2.553		B	
Conc.*N source / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:							
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant		
5 mM *NH4Cl vs 15 mM *NH4Cl	0.844	7.300	3.558	< 0.0001	Yes		
5 mM *NH4Cl vs 5 mM *NH4NO3	0.738	6.378	3.558	0.000	Yes		
5 mM *NH4Cl vs 15 mM *NH4NO3	0.736	6.359	3.558	0.000	Yes		
5 mM *NH4Cl vs 15 mM *KNO3	0.638	5.514	3.558	0.001	Yes		
5 mM *NH4Cl vs 1 mM *NH4Cl	0.558	4.822	3.558	0.004	Yes		
5 mM *NH4Cl vs 5 mM *KNO3	0.376	3.247	3.558	0.088	No		
5 mM *NH4Cl vs 1 mM *NH4NO3	0.333	2.882	3.558	0.166	No		
5 mM *NH4Cl vs 1 mM *KNO3	0.100	0.865	3.558	0.992	No		
1 mM *KNO3 vs 15 mM *NH4Cl	0.744	6.436	3.558	0.000	Yes		

1 mM *KNO3 vs 5 mM *NH4NO3	0.638	5.514	3.558	0.001	Yes		
1 mM *KNO3 vs 15 mM *NH4NO3	0.636	5.494	3.558	0.001	Yes		
1 mM *KNO3 vs 15 mM *KNO3	0.538	4.649	3.558	0.006	Yes		
1 mM *KNO3 vs 1 mM *NH4Cl	0.458	3.958	3.558	0.023	Yes		
1 mM *KNO3 vs 5 mM *KNO3	0.276	2.382	3.558	0.354	No		
1 mM *KNO3 vs 1 mM *NH4NO3	0.233	2.017	3.558	0.554	No		
1 mM *NH4NO3 vs 15 mM *NH4Cl	0.511	4.419	3.558	0.010	Yes		
1 mM *NH4NO3 vs 5 mM *NH4NO3	0.404	3.496	3.558	0.056	No		
1 mM *NH4NO3 vs 15 mM *NH4NO3	0.402	3.477	3.558	0.058	No		
1 mM *NH4NO3 vs 15 mM *KNO3	0.304	2.632	3.558	0.247	No		
1 mM *NH4NO3 vs 1 mM *NH4Cl	0.224	1.940	3.558	0.599	No		
1 mM *NH4NO3 vs 5 mM *KNO3	0.042	0.365	3.558	1.000	No		
5 mM *KNO3 vs 15 mM *NH4Cl	0.469	4.054	3.558	0.020	Yes		
5 mM *KNO3 vs 5 mM *NH4NO3	0.362	3.131	3.558	0.108	No		
5 mM *KNO3 vs 15 mM *NH4NO3	0.360	3.112	3.558	0.112	No		
5 mM *KNO3 vs 15 mM *KNO3	0.262	2.267	3.558	0.413	No		
5 mM *KNO3 vs 1 mM *NH4Cl	0.182	1.575	3.558	0.804	No		
1 mM *NH4Cl vs 15 mM *NH4Cl	0.287	2.478	3.558	0.310	No		
1 mM *NH4Cl vs 5 mM *NH4NO3	0.180	1.556	3.558	0.814	No		
1 mM *NH4Cl vs 15 mM *NH4NO3	0.178	1.537	3.558	0.823	No		
1 mM *NH4Cl vs 15 mM *KNO3	0.080	0.692	3.558	0.998	No		
15 mM *KNO3 vs 15 mM *NH4Cl	0.207	1.787	3.558	0.690	No		
15 mM *KNO3 vs 5 mM *NH4NO3	0.100	0.865	3.558	0.992	No		
15 mM *KNO3 vs 15 mM *NH4NO3	0.098	0.845	3.558	0.993	No		
15 mM *NH4NO3 vs 15 mM *NH4Cl	0.109	0.941	3.558	0.986	No		
15 mM *NH4NO3 vs 5 mM *NH4NO3	0.002	0.019	3.558	1.000	No		
5 mM *NH4NO3 vs 15 mM *NH4Cl	0.107	0.922	3.558	0.988	No		
Tukey's d critical value:			5.031				
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
5 mM *NH4Cl	3.056	0.082	2.882	3.229	A		
1 mM *KNO3	2.956	0.082	2.782	3.129	A		
1 mM *NH4NO3	2.722	0.082	2.549	2.896	A	B	
5 mM *KNO3	2.680	0.082	2.507	2.853	A	B	
1 mM *NH4Cl	2.498	0.082	2.324	2.671		B	C
15 mM *KNO3	2.418	0.082	2.244	2.591		B	C
15 mM *NH4NO3	2.320	0.082	2.147	2.493		B	C
5 mM *NH4NO3	2.318	0.082	2.144	2.491		B	C
15 mM *NH4Cl	2.211	0.082	2.038	2.385			C

2.2 Four applications of INCYDE with 1-10 mM KNO_3

2.2.1 Four applications of INCYDE (0.1 mM KNO_3): Shoot DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	61.09	30.54	0.49	0.673
Treatment	1	68.14	68.14	1.09	0.407
Error	2	125.60	62.80		
Total	5	254.83			

2.2.2 Four applications of INCYDE (0.1 mM KNO_3): Silique number

Poisson Regression Analysis: No. Of siliques versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	0.02969	12.58%	0.02969	0.009897	0.03	0.999
Block	2	0.02969	12.58%	0.02969	0.014845	0.03	0.985
Treatment	1	*	*	*	*	*	*
Error	2	0.20632	87.42%	0.20632	0.103162		
Total	5	0.23602	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	-0.41	1.01	(-2.39, 1.58)	-0.40	0.689	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	-0.11	1.26	(-2.57, 2.36)	-0.08	0.933	1.27
Block3	-0.22	1.30	(-2.77, 2.32)	-0.17	0.864	1.27
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	-0.00	1.05	(-2.07, 2.07)	-0.00	1.000	1.00

Regression Equation

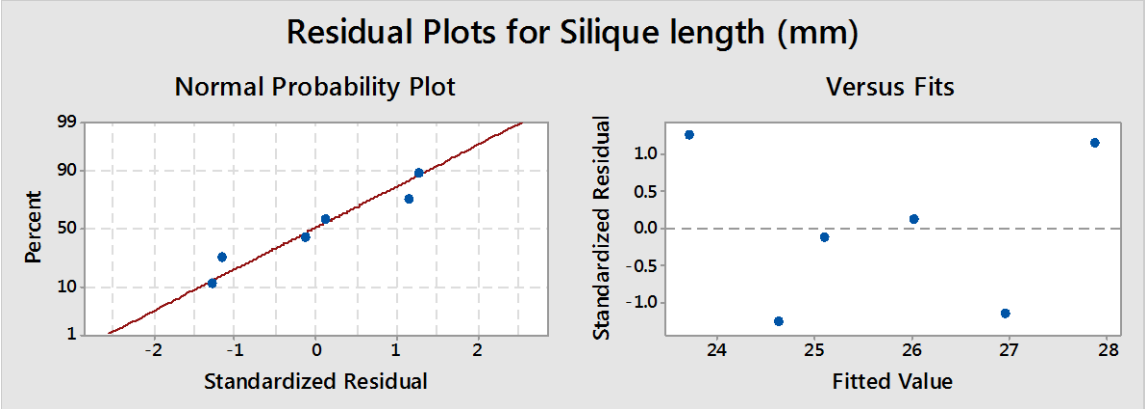
No. Of siliques = exp(Y')

Y' = -0.41 +0.0 Block_Block1 -0.11Block_Block2 -0.22Block_Block3
+0.0Treatment_Control -0.00Treatment_INCYDE 25 µM

Goodness-of*F*-Fit Tests

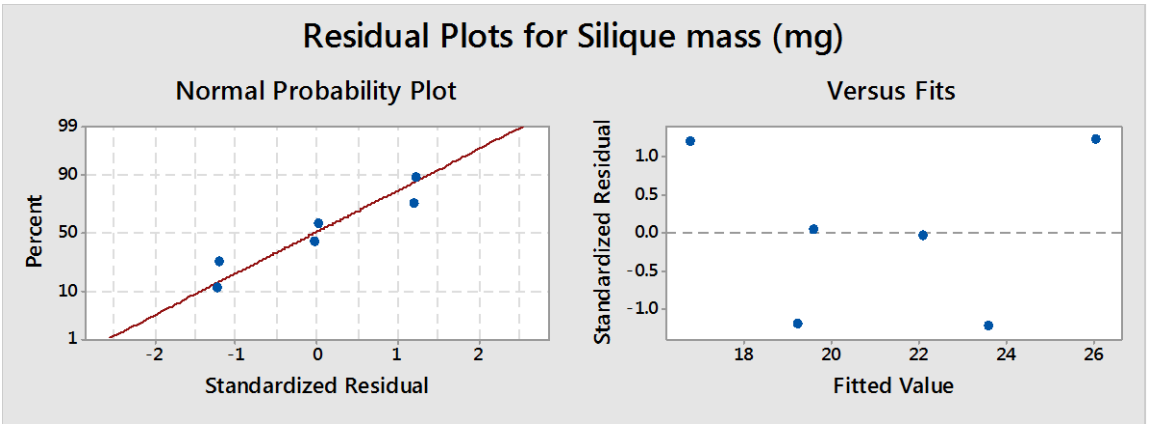
Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	2	0.20632	0.10316	0.21	0.902
Pearson	2	0.20333	0.10167	0.20	0.903

2.2.3 Four applications of INCYDE (0.1 mM KNO_3): Silique length



Analysis of Variance					
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	10.649	5.324	2.16	0.316
Treatment	1	1.241	1.241	0.50	0.551
Error	2	4.925	2.463		
Total	5	16.815			

2.2.4 Four applications of INCYDE (0.1 mM KNO_3): Silique mass



Analysis of Variance					
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	46.605	23.3025	30.92	0.031
Treatment	1	9.149	9.1487	12.14	0.073
Error	2	1.507	0.7536		
Total	5	57.261			

2.2.5 Four applications of INCYDE (0.1 mM KNO_3): Seeds per silique

```
Poisson Regression Analysis: Seeds/silique versus Block, Treatment

Deviance Table
Source       DF  Seq Dev  Contribution  Adj Dev  Adj Mean  Chi-Square  P-Value
Regression    3  0.68715        92.93%  0.68715   0.22905        0.69    0.876
  Block       2  0.59712        80.75%  0.59712   0.29856        0.60    0.742
  Treatment   1  0.09003        12.18%  0.09003   0.09003        0.09    0.764
Error         2  0.05230         7.07%  0.05230   0.02615
Total         5  0.73945       100.00%

Coefficients
Term                Coef   SE Coef         95% CI         Z-Value  P-Value   VIF
Constant           1.672     0.354  (   0.979,    2.366)     4.73    0.000
Block
  Block1         0.000000  0.000000  (0.000000, 0.000000)        *        *     *
  Block2            0.286     0.416  (  -0.529,    1.101)     0.69    0.491  1.41
  Block3            0.026     0.441  (  -0.839,    0.892)     0.06    0.952  1.41
Treatment
  Control       0.000000  0.000000  (0.000000, 0.000000)        *        *     *
  INCYDE 25 µM    -0.103     0.343  (  -0.776,    0.570)    -0.30    0.764  1.00

Regression Equation
Seeds/silique  =  exp(Y')
Y' = 1.672 +0.0Block_Block1 +0.286Block_Block2 +0.026Block_Block3
     +0.0Treatment_Control  -0.103Treatment_INCYDE 25 µM

Goodness-of-Fit Tests
Test      DF  Estimate     Mean  Chi-Square  P-Value
Deviance   2   0.05230  0.02615        0.05    0.974
Pearson    2   0.05221  0.02611        0.05    0.974
```

2.2.6 Four applications of INCYDE (0.1 mM KNO_3): Seeds per plant

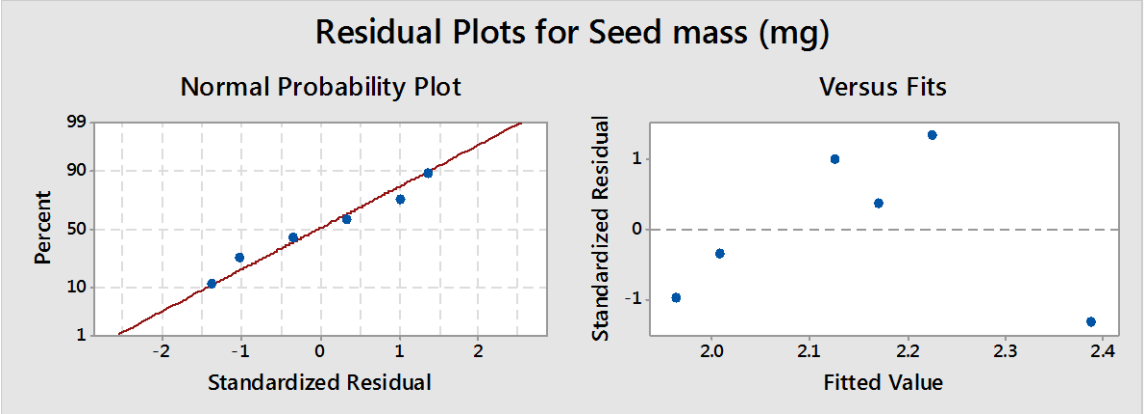
```
Poisson Regression Analysis: Seeds/plant versus Block, Treatment

Deviance Table
Source       DF  Seq Dev  Contribution  Adj Dev  Adj Mean  Chi-Square  P-Value
Regression    3  0.60021        28.83%  0.60021  0.200070        0.60    0.896
  Block       2  0.59949        28.79%  0.59949  0.299745        0.60    0.741
  Treatment   1  0.00072         0.03%  0.00072  0.000721        0.00    0.979
Error         2  1.48180        71.17%  1.48180  0.740899
Total         5  2.08201       100.00%

Coefficients
Term                Coef   SE Coef         95% CI         Z-Value  P-Value   VIF
Constant           1.245     0.440  (   0.382,    2.108)     2.83    0.005
Block
  Block1         0.000000  0.000000  (0.000000, 0.000000)        *        *     *
  Block2            0.150     0.519  (  -0.867,    1.168)     0.29    0.773  1.30
  Block3           -0.277     0.580  (  -1.414,    0.859)    -0.48    0.632  1.30
Treatment
  Control       0.000000  0.000000  (0.000000, 0.000000)        *        *     *
  INCYDE 25 µM    -0.012     0.445  (  -0.885,    0.861)    -0.03    0.979  1.00

Regression Equation
Seeds/plant  =  exp(Y')
Y' = 1.245 +0.0Block_Block1 +0.150Block_Block2 -0.277Block_Block3
     +0.0Treatment_Control  -0.012Treatment_INCYDE 25 µM
```

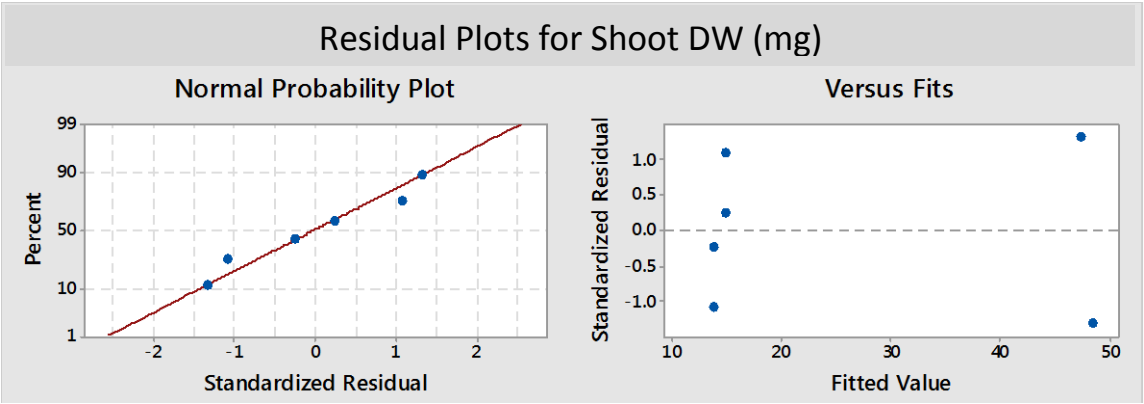
2.2.7 Four applications of INCYDE (0.1 mM KNO_3): Seed mass



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.08005	0.04002	1.32	0.430
Treatment	1	0.04002	0.04002	1.32	0.369
Error	2	0.06048	0.03024		
Total	5	0.18054			

2.2.8 Four applications of INCYDE (1 mM KNO_3): Shoot DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	1500.10	750.049	249.56	0.004
Treatment	1	1.71	1.706	0.57	0.530
Error	2	6.01	3.005		
Total	5	1507.81			

2.2.9 Four applications of INCYDE (1 mM KNO_3): Silique number

Poisson Regression Analysis: No. Of siliques versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	1.81747	94.03%	1.81747	0.60582	1.82	0.611
Block	2	1.78122	92.16%	1.78122	0.89061	1.78	0.410
Treatment	1	0.03625	1.88%	0.03625	0.03625	0.04	0.849
Error	2	0.11536	5.97%	0.11536	0.05768		
Total	5	1.93283	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	0.289	0.684	(-1.051, 1.630)	0.42	0.672	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	0.314	0.827	(-1.306, 1.934)	0.38	0.704	1.70
Block3	0.906	0.745	(-0.554, 2.365)	1.22	0.224	1.70
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	-0.109	0.572	(-1.230, 1.012)	-0.19	0.849	1.00

Regression Equation

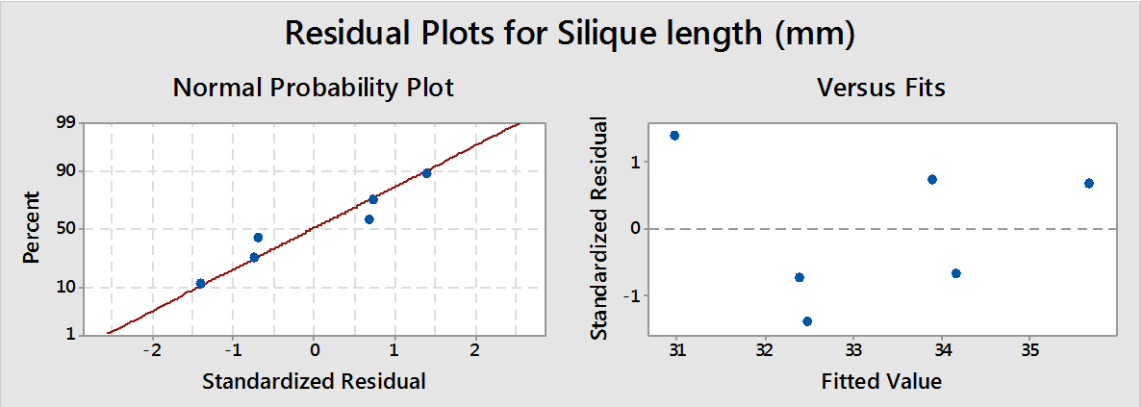
No. Of siliques = exp(Y')

Y' = 0.289 +0.0Block_Block1 +0.314Block_Block2 +0.906Block_Block3
+0.0Treatment_Control -0.109Treatment_INCYDE 25 µM

Goodness-of*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	2	0.11536	0.05768	0.12	0.944
Pearson	2	0.11482	0.05741	0.11	0.944

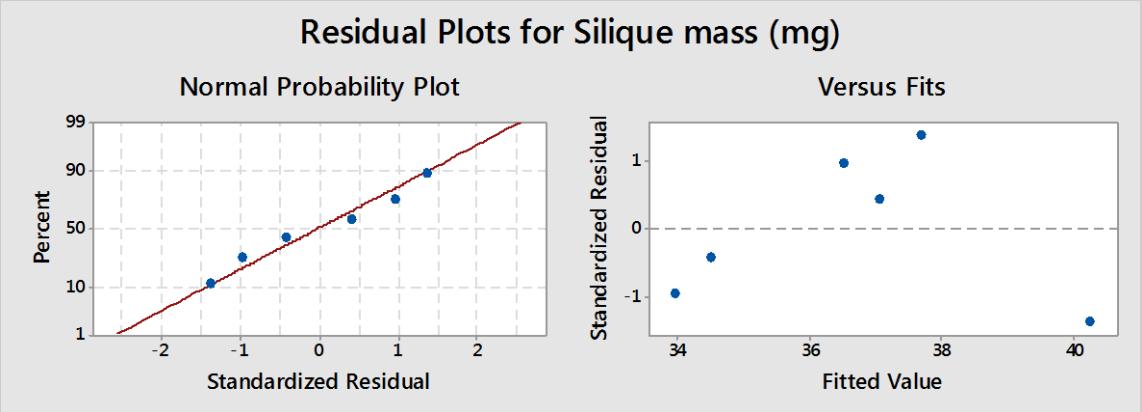
2.2.10 Four applications of INCYDE (1 mM KNO_3): Silique length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	10.388	5.194	0.99	0.503
Treatment	1	3.425	3.425	0.65	0.504
Error	2	10.518	5.259		
Total	5	24.331			

2.2.11 Four applications of INCYDE (1 mM KNO_3): Silique mass (mg)



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	16.395	8.198	0.27	0.788
Treatment	1	9.874	9.874	0.32	0.627
Error	2	61.111	30.556		
Total	5	87.380			

2.2.12 Four applications of INCYDE (1 mM KNO_3): Seeds per silique

Poisson Regression Analysis: Seeds/silique versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	0.6584	82.93%	0.6584	0.21948	0.66	0.883
Block	2	0.1043	13.14%	0.1043	0.05215	0.10	0.949
Treatment	1	0.5541	69.79%	0.5541	0.55413	0.55	0.457
Error	2	0.1355	17.07%	0.1355	0.06776		
Total	5	0.7940	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	2.281	0.257	(1.778, 2.785)	8.89	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	-0.065	0.309	(-0.671, 0.541)	-0.21	0.833	1.30
Block3	-0.099	0.312	(-0.710, 0.512)	-0.32	0.752	1.30
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	0.190	0.256	(-0.312, 0.692)	0.74	0.458	1.00

Regression Equation

Seeds/silique = exp(Y')

Y' = 2.281 +0.0Block_Block1 -0.065Block_Block2 -0.099Block_Block3
+0.0Treatment_Control +0.190Treatment_INCYDE 25 µM

Goodness-o*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	2	0.13553	0.06776	0.14	0.934
Pearson	2	0.13525	0.06762	0.14	0.935

2.2.13 Four applications of INCYDE (1 mM KNO_3): Seeds per plant

```
Poisson Regression Analysis: Seeds/plant versus Block, Treatment

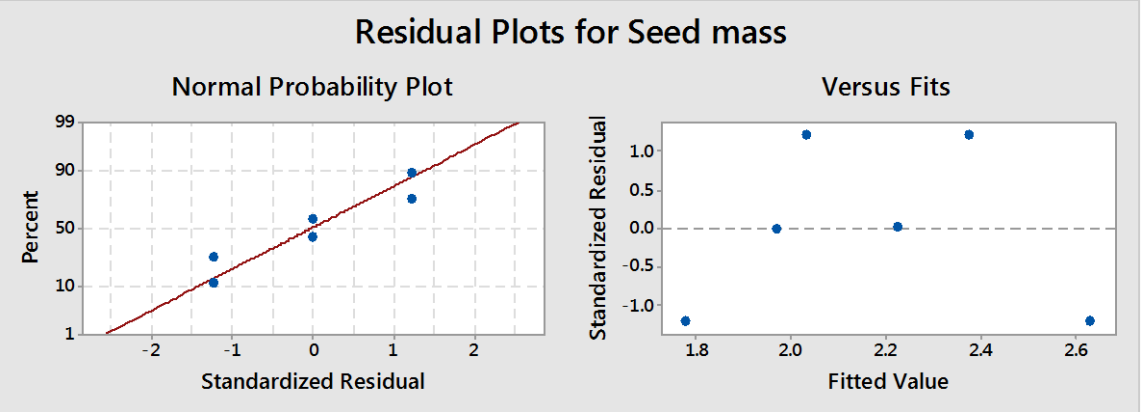
Deviance Table
Source        DF  Seq Dev  Contribution  Adj Dev  Adj Mean  Chi-Square  P-Value
Regression     3  15.3640        85.15%  15.3640    5.1213       15.36    0.002
  Block        2  15.0095        83.19%  15.0095    7.5047       15.01    0.001
  Treatment    1   0.3546         1.97%   0.3546    0.3546        0.35    0.552
Error          2   2.6789        14.85%   2.6789    1.3395
Total          5  18.0429       100.00%

Coefficients
Term                 Coef   SE Coef          95% CI          Z-Value  P-Value   VIF
Constant            2.534     0.216  (   2.111,    2.958)    11.73    0.000
Block
  Block1         0.000000  0.000000  (0.000000, 0.000000)        *        *     *
  Block2            0.245     0.259  (  -0.262,    0.752)     0.95    0.344  1.64
  Block3            0.819     0.232  (   0.363,    1.275)     3.52    0.000  1.64
Treatment
  Control        0.000000  0.000000  (0.000000, 0.000000)        *        *     *
  INCYDE 25 µM      0.108     0.182  (  -0.248,    0.465)     0.60    0.552  1.00

Regression Equation
Seeds/plant  =  exp(Y')
Y' = 2.534 +0.0Block_Block1 +0.245Block_Block2 +0.819Block_Block3
     +0.0Treatment_Control  +0.108Treatment_INCYDE 25 µM
Model Summary

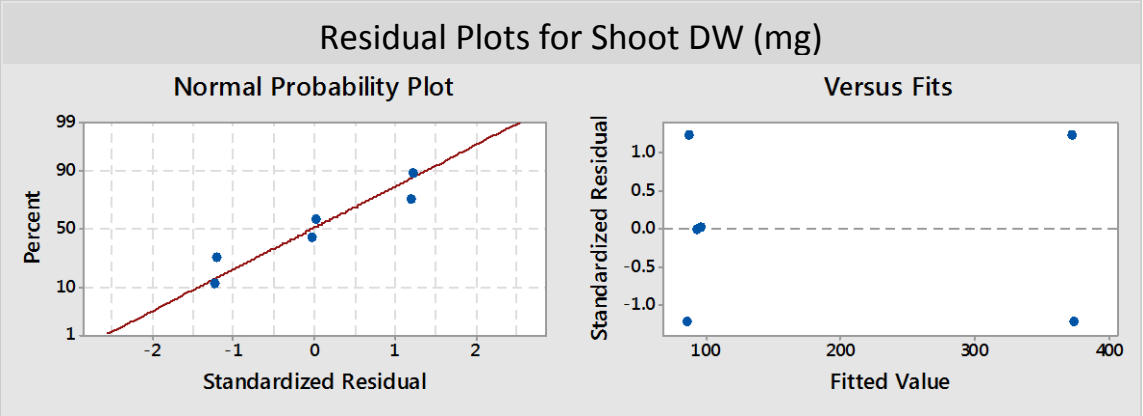
Deviance
    R-Sq
  85.15%
```

2.2.14 Four applications of INCYDE (1 mM KNO_3): Seed mass




```
Analysis of Variance
Source        DF   Adj SS   Adj MS  F-Value  P-Value
  Block        2  0.37118  0.18559     6.82    0.128
  Treatment    1  0.09711  0.09711     3.57    0.200
Error          2  0.05445  0.02722
Total          5  0.52274
```

2.2.15 Four applications of INCYDE (5 mM KNO_3): Shoot DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	106888	53444.0	1134.62	0.001
Treatment	1	8	7.8	0.17	0.723
Error	2	94	47.1		
Total	5	106990			

2.2.16 Four applications of INCYDE (5 mM KNO_3): Silique number

Poisson Regression Analysis: No. Of siliques versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	7.06922	99.31%	7.06922	2.35641	7.07	0.070
Block	2	6.29548	88.44%	6.29548	3.14774	6.30	0.043
Treatment	1	0.77374	10.87%	0.77374	0.77374	0.77	0.379
Error	2	0.04887	0.69%	0.04887	0.02444		
Total	5	7.11810	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	1.208	0.406	(0.413, 2.003)	2.98	0.003	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	0.154	0.487	(-0.801, 1.108)	0.32	0.753	1.62
Block3	0.910	0.423	(0.081, 1.740)	2.15	0.031	1.62
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	0.293	0.335	(-0.363, 0.949)	0.87	0.382	1.00

Regression Equation

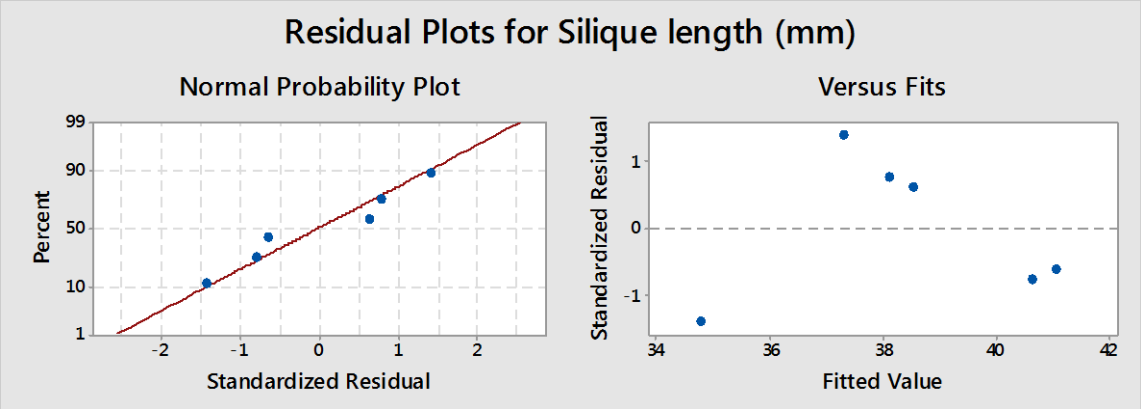
No. Of siliques = exp(Y')

Y' = 1.208 +0.0Block_Block1 +0.154Block_Block2 +0.910Block_Block3
+0.0Treatment_Control +0.293Treatment_INCYDE 25 µM

Goodness-of-*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	2	0.04887	0.02444	0.05	0.976
Pearson	2	0.04892	0.02446	0.05	0.976

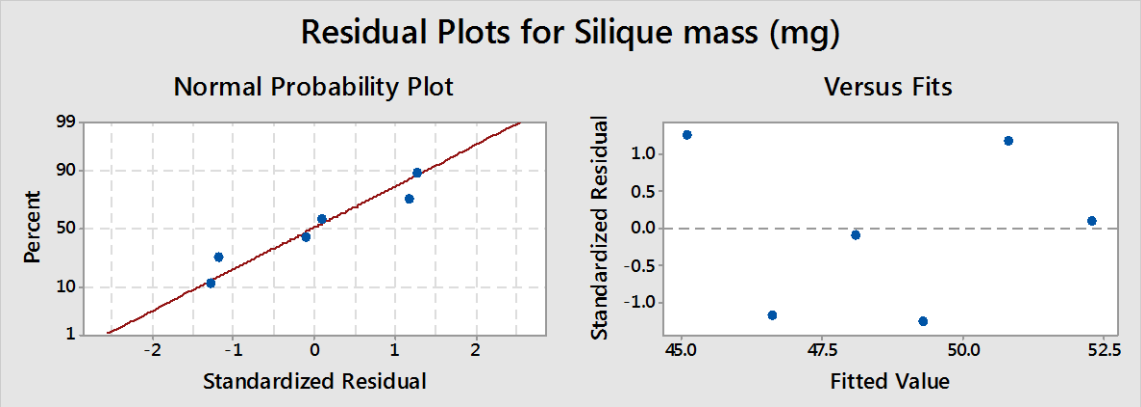
2.2.17 Four applications of INCYDE (5 mM KNO_3): Silique length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	17.056	8.5280	9.23	0.098
Treatment	1	9.627	9.6267	10.42	0.084
Error	2	1.848	0.9239		
Total	5	28.530			

2.2.18 Four applications of INCYDE (5 mM KNO_3): Silique mass



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	8.971	4.486	0.35	0.740
Treatment	1	26.488	26.488	2.08	0.286
Error	2	25.480	12.740		
Total	5	60.939			

2.2.19 Four applications of INCYDE (5 mM KNO_3): Seeds per silique

Poisson Regression Analysis: Seeds/silique versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	3.70161	84.71%	3.70161	1.23387	3.70	0.296
Block	2	3.68493	84.33%	3.68493	1.84247	3.68	0.158
Treatment	1	0.01667	0.38%	0.01667	0.01667	0.02	0.897
Error	2	0.66814	15.29%	0.66814	0.33407		
Total	5	4.36975	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	2.648	0.221	(2.214, 3.083)	11.96	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	-0.057	0.268	(-0.582, 0.468)	-0.21	0.830	1.22
Block3	-0.538	0.307	(-1.140, 0.065)	-1.75	0.080	1.22
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	0.030	0.235	(-0.430, 0.491)	0.13	0.897	1.00

Regression Equation

Seeds/silique = exp(Y')

Y' = 2.648 +0.0Block_Block1 -0.057Block_Block2 -0.538Block_Block3
+0.0Treatment_Control +0.030Treatment_INCYDE 25 µM

Model Summary

Deviance R-Sq
84.71%

2.2.20 Four applications of INCYDE (5 mM KNO_3): Seeds per plant

Poisson Regression Analysis: Seeds/plant versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	15.6479	94.00%	15.6479	5.2160	15.65	0.001
Block	2	6.1937	37.21%	6.1937	3.0969	6.19	0.045
Treatment	1	9.4542	56.79%	9.4542	9.4542	9.45	0.002
Error	2	0.9993	6.00%	0.9993	0.4997		
Total	5	16.6472	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	3.877	0.111	(3.659, 4.094)	34.97	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	0.049	0.131	(-0.207, 0.305)	0.37	0.708	1.41
Block3	0.284	0.124	(0.041, 0.526)	2.29	0.022	1.41
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	0.315	0.103	(0.113, 0.517)	3.06	**0.002**	1.00

Regression Equation

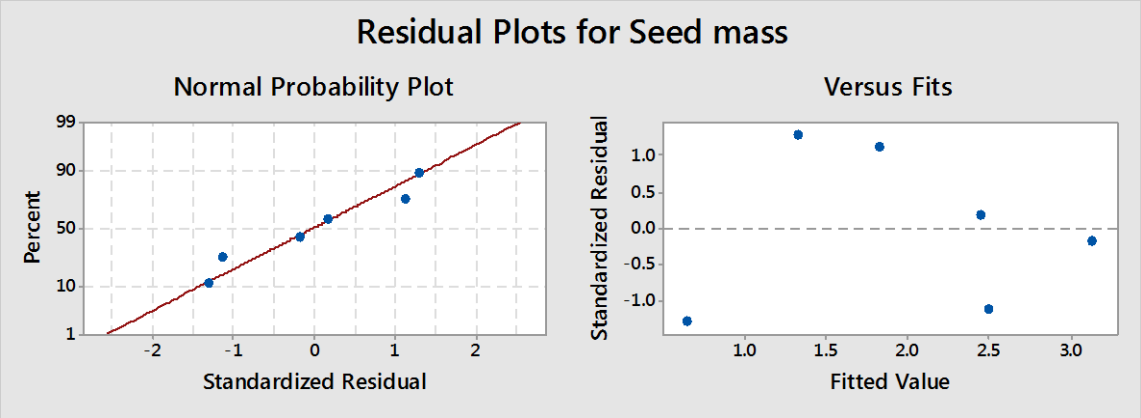
Seeds/plant = exp(Y')

Y' = 3.877 +0.0Block_Block1 +0.049Block_Block2 +0.284Block_Block3
+0.0Treatment_Control +0.315Treatment_INCYDE 25 µM

Model Summary

Deviance R-Sq
94.00%

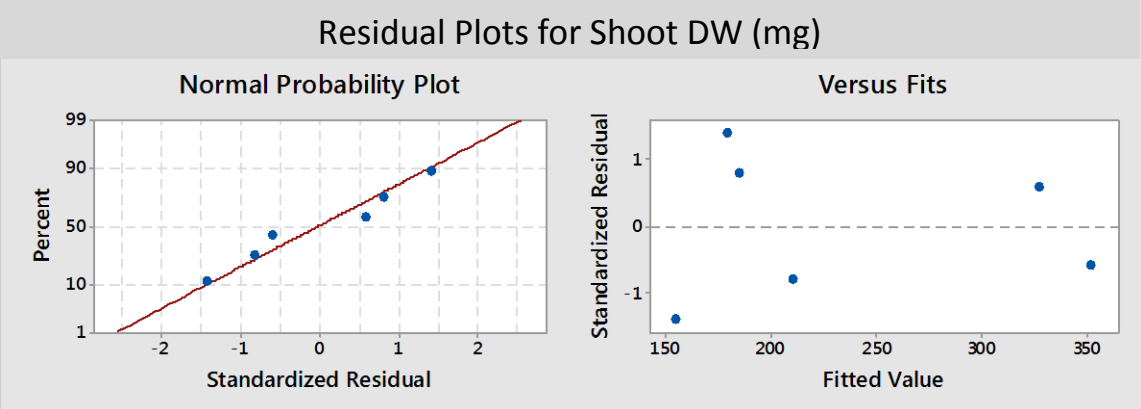
2.2.21 Four applications of INCYDE (5 mM KNO_3): Seed mass



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	3.3222	1.6611	3.67	0.214
Treatment	1	0.6913	0.6913	1.53	0.342
Error	2	0.9061	0.4531		
Total	5	4.9197			

2.2.22 Four applications of INCYDE (10 mM KNO_3): Shoot DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	33858.9	16929.4	90.43	0.011
Treatment	1	964.4	964.4	5.15	0.151
Error	2	374.4	187.2		
Total	5	35197.7			

2.2.23 Four applications of INCYDE (10 mM KNO_3): Silique number

Poisson Regression Analysis: No. Of siliques versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	1.2203	89.64%	1.2203	0.40678	1.22	0.748
Block	2	1.0788	79.25%	1.0788	0.53942	1.08	0.583
Treatment	1	0.1415	10.39%	0.1415	0.14150	0.14	0.707
Error	2	0.1410	10.36%	0.1410	0.07049		
Total	5	1.3613	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	1.680	0.351	(0.992, 2.368)	4.78	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	0.103	0.434	(-0.747, 0.954)	0.24	0.812	1.46
Block3	0.398	0.407	(-0.400, 1.195)	0.98	0.328	1.46
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	-0.125	0.332	(-0.777, 0.527)	-0.38	0.707	1.00

Regression Equation

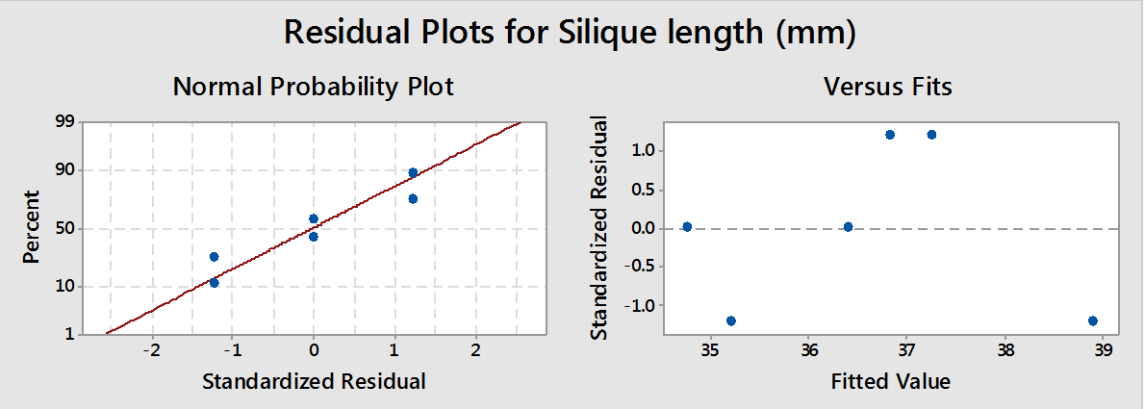
No. Of siliques = exp(Y')

Y' = 1.680 +0.0Block_Block1 +0.103Block_Block2 +0.398Block_Block3
+0.0Treatment_Control -0.125Treatment_INCYDE 25 µM

Goodness-of*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	2	0.14098	0.07049	0.14	0.932
Pearson	2	0.14096	0.07048	0.14	0.932

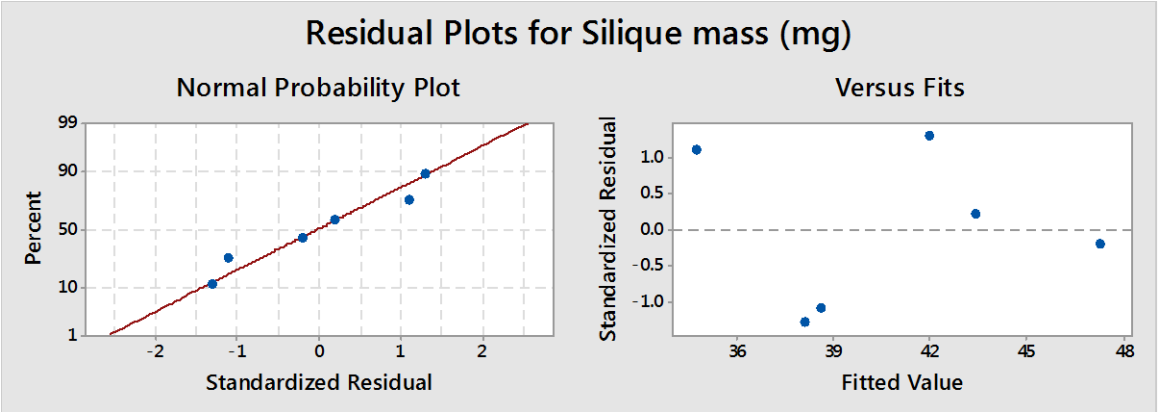
2.2.24 Four applications of INCYDE (10 mM KNO_3): Silique length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	7.139	3.570	0.43	0.698
Treatment	1	4.002	4.002	0.48	0.559
Error	2	16.538	8.269		
Total	5	27.679			

2.2.25 Four applications of INCYDE (10 mM KNO_3): Silique mass



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	76.32	38.16	0.67	0.600
Treatment	1	22.66	22.66	0.40	0.593
Error	2	114.33	57.17		
Total	5	213.31			

2.2.26 Four applications of INCYDE (10 mM KNO_3): Seeds per silique

Poisson Regression Analysis: Seeds/silique versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	3.85589	78.63%	3.85589	1.28530	3.86	0.277
Block	2	3.83584	78.22%	3.83584	1.91792	3.84	0.147
Treatment	1	0.02004	0.41%	0.02004	0.02004	0.02	0.887
Error	2	1.04792	21.37%	1.04792	0.52396		
Total	5	4.90380	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	2.717	0.215	(2.296, 3.139)	12.63	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	-0.042	0.262	(-0.555, 0.471)	-0.16	0.872	1.22
Block3	-0.530	0.301	(-1.120, 0.060)	-1.76	0.078	1.22
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	-0.033	0.230	(-0.483, 0.418)	-0.14	0.887	1.00

Regression Equation

Seeds/silique = exp(Y')

Y' = 2.717 +0.0Block_Block1 -0.042Block_Block2 -0.530Block_Block3
+0.0Treatment_Control -0.033Treatment_INCYDE 25 µM

Model Summary

Deviance R-Sq
78.63%

2.2.27 Four applications of INCYDE (10 mM KNO_3): Seeds per plant

Poisson Regression Analysis: Seeds/plant versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	0.94666	94.80%	0.94666	0.31555	0.95	0.814
Block	2	0.39520	39.58%	0.39520	0.19760	0.40	0.821
Treatment	1	0.55146	55.22%	0.55146	0.55146	0.55	0.458
Error	2	0.05193	5.20%	0.05193	0.02596		
Total	5	0.99859	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	4.3558	0.0932	(4.1731, 4.5386)	46.72	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	0.043	0.114	(-0.181, 0.266)	0.38	0.707	1.34
Block3	-0.029	0.116	(-0.256, 0.199)	-0.25	0.804	1.34
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	-0.0697	0.0939	(-0.2538, 0.1143)	-0.74	0.458	1.00

Regression Equation

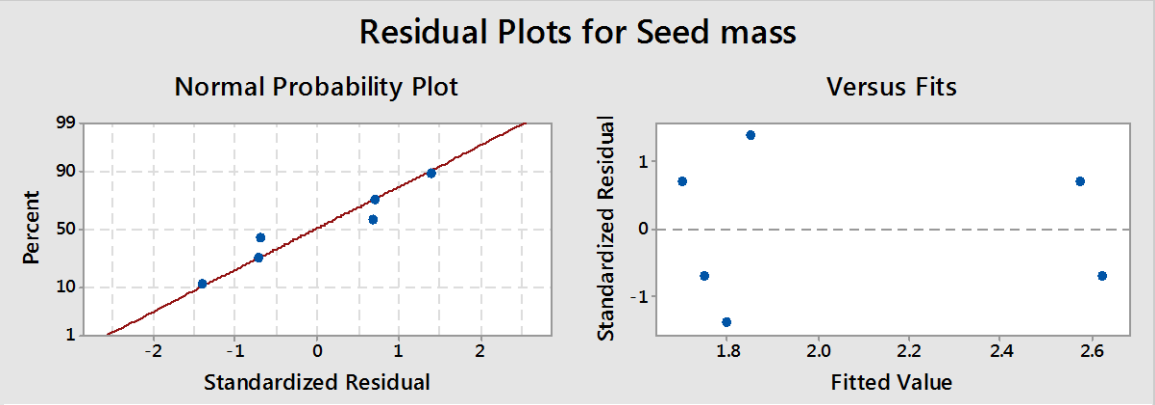
Seeds/plant = exp(Y')

Y' = 4.3558 +0.0Block_Block1 +0.043Block_Block2 -0.029Block_Block3
+0.0Treatment_Control -0.0697Treatment_INCYDE 25 µM

Goodness-o*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	2	0.05193	0.02596	0.05	0.974
Pearson	2	0.05192	0.02596	0.05	0.974

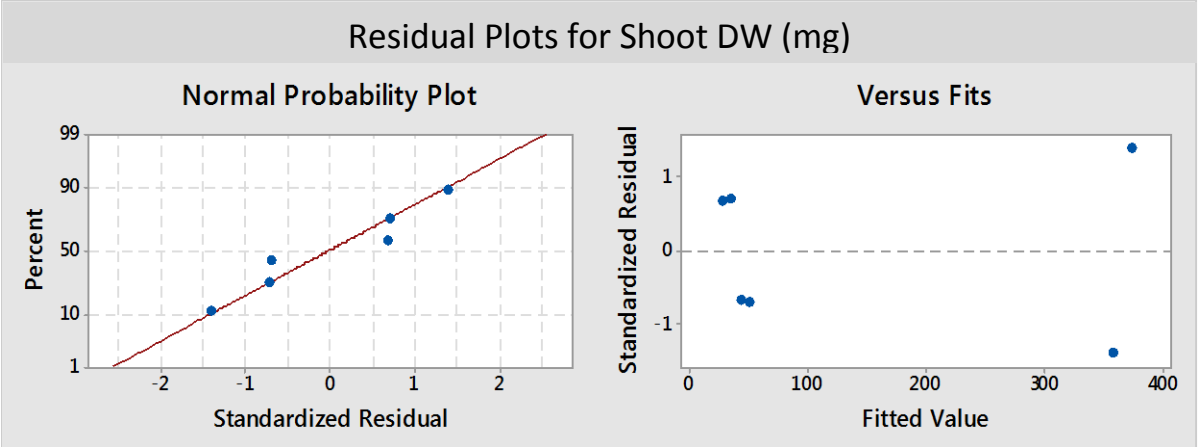
2.2.28 Four applications of INCYDE (10 mM KNO_3): Seed mass



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	76.32	38.16	0.67	0.600
Treatment	1	22.66	22.66	0.40	0.593
Error	2	114.33	57.17		
Total	5	213.31			

2.2.29 Four applications of INCYDE (fertiliser): Shoot DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	143725	71862.4	176.01	0.006
Treatment	1	367	367.3	0.90	0.443
Error	2	817	408.3		
Total	5	144909			

2.2.30 Four applications of INCYDE (fertiliser): Silique number

Poisson Regression Analysis: No. Of siliques versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	7.81414	98.59%	7.81414	2.60471	7.81	0.050
Block	2	7.78078	98.17%	7.78078	3.89039	7.78	0.020
Treatment	1	0.03335	0.42%	0.03335	0.03335	0.03	0.855
Error	2	0.11155	1.41%	0.11155	0.05577		
Total	5	7.92569	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	1.198	0.424	(0.368, 2.028)	2.83	0.005	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	-0.017	0.543	(-1.081, 1.047)	-0.03	0.974	1.56
Block3	0.985	0.448	(0.108, 1.863)	2.20	0.028	1.56
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	0.065	0.354	(-0.630, 0.759)	0.18	0.855	1.00

Regression Equation

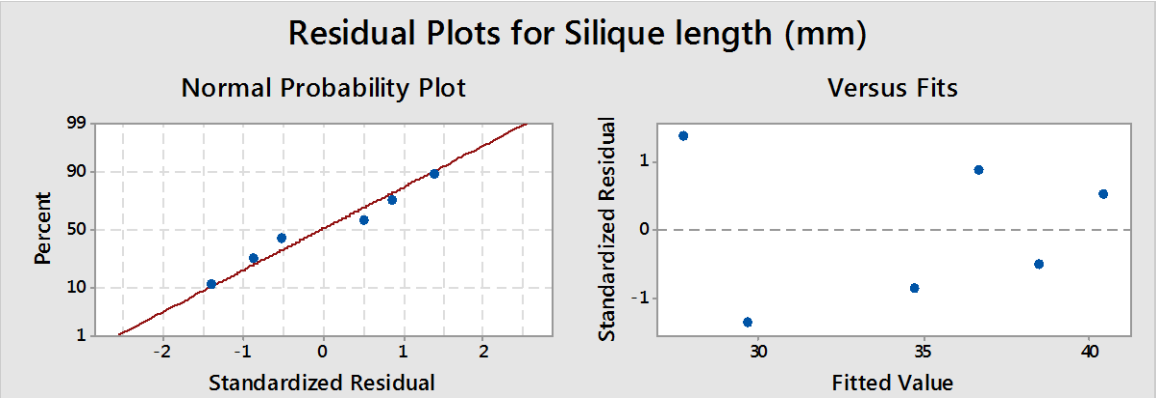
No. Of siliques = exp(Y')

Y' = 1.198 + 0.0Block_Block1 - 0.017Block_Block2 + 0.985Block_Block3
+ 0.0Treatment_Control + 0.065Treatment_INCYDE 25 µM

Goodness-of*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	2	0.11155	0.05577	0.11	0.946
Pearson	2	0.11153	0.05576	0.11	0.946

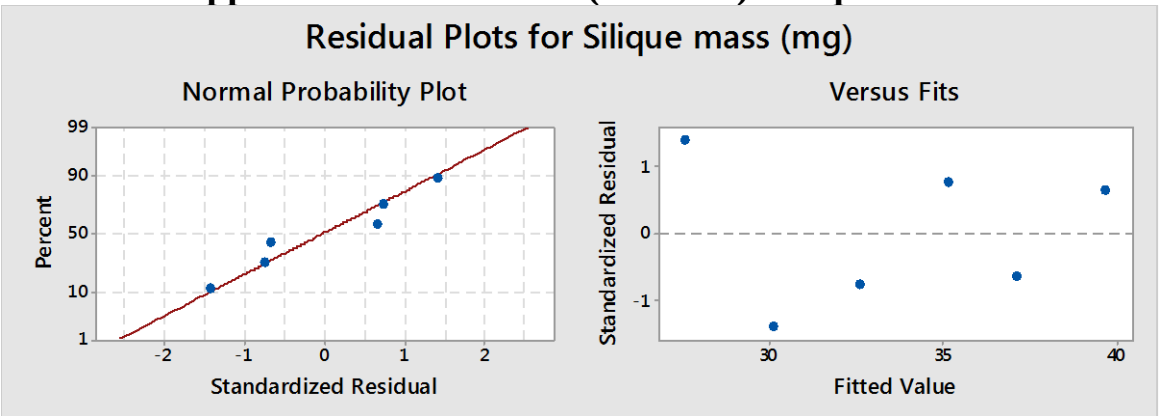
2.2.31 Four applications of INCYDE (fertiliser): Silique length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	120.230	60.115	8.06	0.110
Treatment	1	5.892	5.892	0.79	0.468
Error	2	14.919	7.460		
Total	5	141.042			

2.2.32 Four applications of INCYDE (fertiliser): Silique mass



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	91.813	45.906	2.94	0.254
Treatment	1	9.909	9.909	0.63	0.509
Error	2	31.219	15.610		
Total	5	132.942			

2.2.33 Four applications of INCYDE (fertiliser): Seeds per silique

Poisson Regression Analysis: Seeds/silique versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	1.92731	86.16%	1.92731	0.64244	1.93	0.588
Block	2	1.84157	82.32%	1.84157	0.92078	1.84	0.398
Treatment	1	0.08574	3.83%	0.08574	0.08574	0.09	0.770
Error	2	0.30969	13.84%	0.30969	0.15484		
Total	5	2.23700	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	2.735	0.215	(2.314, 3.156)	12.73	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	-0.223	0.275	(-0.762, 0.315)	-0.81	0.417	1.22
Block3	-0.382	0.288	(-0.946, 0.181)	-1.33	0.184	1.22
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	-0.068	0.233	(-0.524, 0.388)	-0.29	0.770	1.00

Regression Equation

Seeds/silique = exp(Y')

Y' = 2.735 + 0.0Block_Block1 - 0.223Block_Block2 - 0.382Block_Block3
+ 0.0Treatment_Control - 0.068Treatment_INCYDE 25 µM

Model Summary

Deviance R-Sq
89.06%

2.2.34 Four applications of INCYDE (fertiliser): Seeds per plant

Poisson Regression Analysis: Seeds/plant versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	37.7755	89.06%	37.7755	12.5918	37.78	0.000
Block	2	37.7493	88.99%	37.7493	18.8746	37.75	0.000
Treatment	1	0.0262	0.06%	0.0262	0.0262	0.03	0.871
Error	2	4.6419	10.94%	4.6419	2.3210		
Total	5	42.4174	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	3.938	0.112	(3.718, 4.158)	35.05	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	-0.254	0.150	(-0.548, 0.040)	-1.70	0.090	1.38
Block3	0.518	0.125	(0.273, 0.764)	4.14	0.000	1.38
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	-0.017	0.107	(-0.226, 0.192)	-0.16	0.871	1.00

Regression Equation

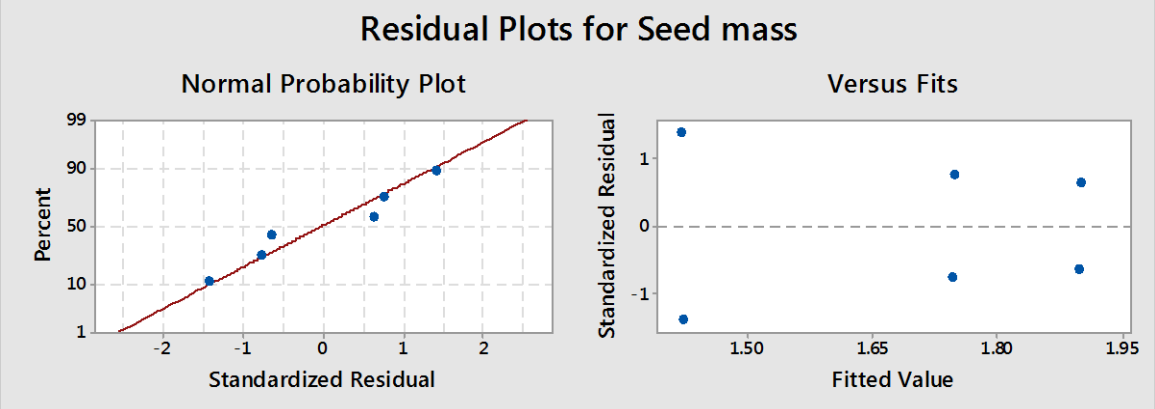
Seeds/plant = exp(Y')

Y' = 3.938 + 0.0Block_Block1 - 0.254Block_Block2 + 0.518Block_Block3
+ 0.0Treatment_Control - 0.017Treatment_INCYDE 25 µM

Model Summary

Deviance R-Sq
89.06%

2.2.35 Four applications of INCYDE (fertiliser): Seed mass



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.238841	0.119421	0.56	0.641
Treatment	1	0.000010	0.000010	0.00	0.995
Error	2	0.425817	0.212908		
Total	5	0.664667			

2.3 Single application of INCYDE

2.3.1 Single application of INCYDE: Silique number

Poisson Regression Analysis: No. siliques versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	2.62148	97.61%	2.62148	0.87383	2.62	0.454
Block	2	2.62085	97.59%	2.62085	1.31043	2.62	0.270
Treatment	1	0.00063	0.02%	0.00063	0.00063	0.00	0.980
Error	2	0.06422	2.39%	0.06422	0.03211		
Total	5	2.68570	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	1.270	0.412	(0.464, 2.077)	3.09	0.002	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	0.686	0.458	(-0.213, 1.584)	1.50	0.135	1.74
Block3	0.579	0.467	(-0.336, 1.495)	1.24	0.215	1.74
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	0.009	0.342	(-0.662, 0.680)	0.03	0.980	1.00

Regression Equation

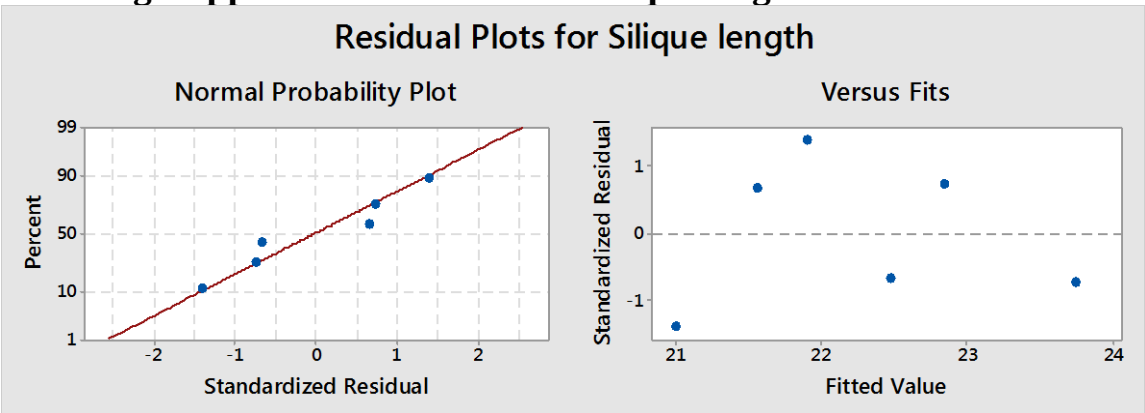
No. siliques = exp(Y')

Y' = 1.270 + 0.0Block_Block1 + 0.686Block_Block2 + 0.579Block_Block3
+ 0.0Treatment_Control + 0.009Treatment_INCYDE 25 µM

Goodness-o*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	2	0.06422	0.03211	0.06	0.968
Pearson	2	0.06418	0.03209	0.06	0.968

2.3.2 Single application of INCYDE: Silique length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	3.594	1.797	0.22	0.819
Treatment	1	1.245	1.245	0.15	0.733
Error	2	16.269	8.135		
Total	5	21.108			

2.3.3 Single application of INCYDE: Seeds per silique

Poisson Regression Analysis: No. seeds/siliques versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	0.140920	18.90%	0.140920	0.046973	0.14	0.987
Block	2	0.140789	18.88%	0.140789	0.070394	0.14	0.932
Treatment	1	0.000131	0.02%	0.000131	0.000131	0.00	0.991
Error	2	0.604642	81.10%	0.604642	0.302321		
Total	5	0.745562	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	1.799	0.335	(1.143, 2.456)	5.37	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	-0.155	0.424	(-0.986, 0.676)	-0.37	0.714	1.29
Block3	-0.045	0.412	(-0.852, 0.762)	-0.11	0.913	1.29
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	-0.004	0.343	(-0.677, 0.669)	-0.01	0.991	1.00

Regression Equation

No. seeds/siliques = exp(Y')

Y' = 1.799 + 0.0Block_Block1 - 0.155Block_Block2 - 0.045Block_Block3
+ 0.0Treatment_Control - 0.004Treatment_INCYDE 25 µM

Goodness-o*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	2	0.60464	0.30232	0.60	0.739
Pearson	2	0.60193	0.30096	0.60	0.740

2.3.4 Single application of INCYDE: Seeds per plant

Poisson Regression Analysis: Seeds/plant versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	4.59324	98.94%	4.59324	1.53108	4.59	0.204
Block	2	4.53855	97.76%	4.53855	2.26928	4.54	0.103
Treatment	1	0.05469	1.18%	0.05469	0.05469	0.05	0.815
Error	2	0.04944	1.06%	0.04944	0.02472		
Total	5	4.64268	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	3.299	0.154	(2.998, 3.600)	21.48	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	0.340	0.176	(-0.005, 0.686)	1.93	0.054	1.43
Block3	0.048	0.188	(-0.321, 0.417)	0.26	0.798	1.43
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
INCYDE 25 µM	0.034	0.145	(-0.250, 0.318)	0.23	0.815	1.00

Regression Equation

Seeds/plant = exp(Y')

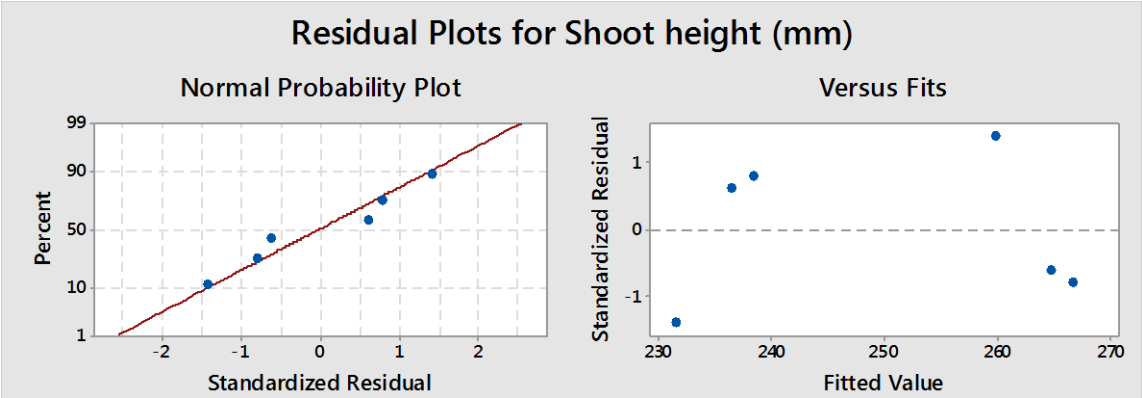
Y' = 3.299 + 0.0Block_Block1 + 0.340Block_Block2 + 0.048Block_Block3
+ 0.0Treatment_Control + 0.034Treatment_INCYDE 25 µM

Goodness-o*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	2	0.04944	0.02472	0.05	0.976
Pearson	2	0.04943	0.02472	0.05	0.976

2.4 Single application of TDZ-K

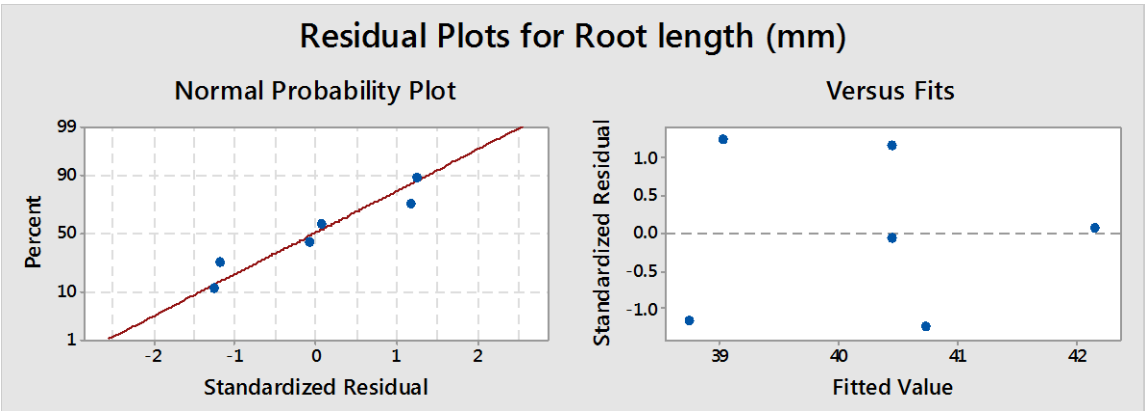
2.4.1 Single application of TDZ-K: Shoot height



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	50.22	25.11	0.34	0.746
Treatment	1	1207.00	1207.00	16.40	0.056
Error	2	147.20	73.60		
Total	5	1404.42			

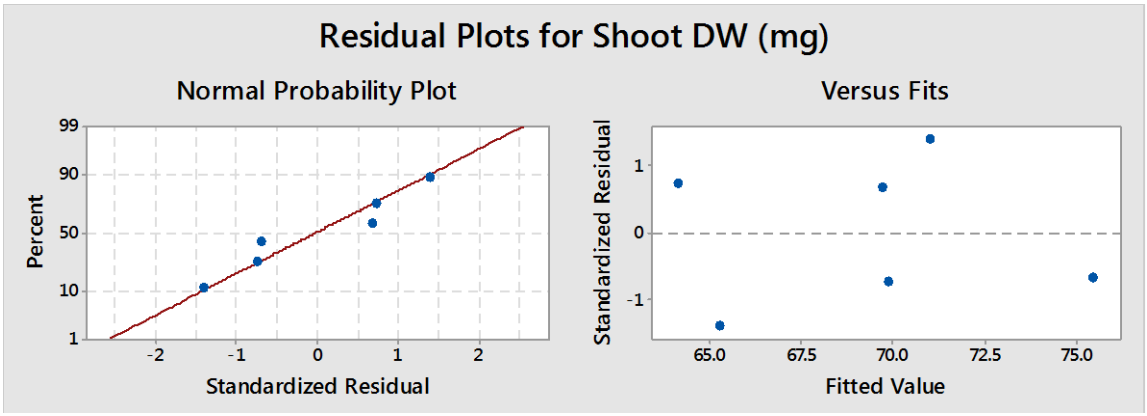
2.4.2 Single application of TDZ-K: Root length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	3.318	1.659	0.26	0.792
Treatment	1	4.335	4.335	0.69	0.495
Error	2	12.643	6.322		
Total	5	20.296			

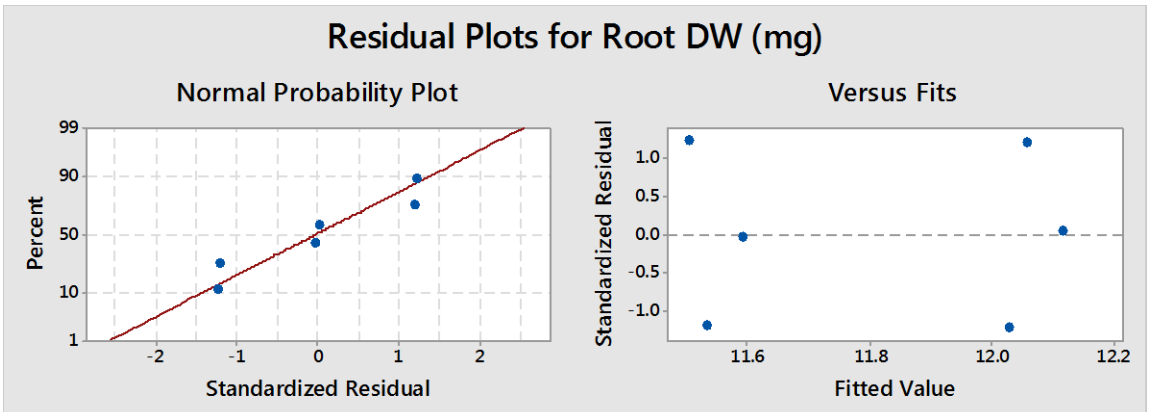
2.4.3 Single application of TDZ-K: Shoot DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	35.087	17.543	3.77	0.210
Treatment	1	49.757	49.757	10.68	0.082
Error	2	9.318	4.659		
Total	5	94.161			

2.4.4 Single application of TDZ-K: Root DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.008190	0.004095	0.07	0.935
Treatment	1	0.413045	0.413045	6.99	0.118
Error	2	0.118249	0.059124		
Total	5	0.539484			

2.4.5 Single application of TDZ-K: Silique number

Poisson Regression Analysis: Silique no./plant versus Block, Treatment

Method

Link function	Natural log
Categorical predictor coding	(1, 0)
Rows used	6

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	0.47121	84.86%	0.47121	0.15707	0.47	0.925
Block	2	0.29836	53.73%	0.29836	0.14918	0.30	0.861
Treatment	1	0.17285	31.13%	0.17285	0.17285	0.17	0.678
Error	2	0.08406	15.14%	0.08406	0.04203		
Total	5	0.55527	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	1.614	0.365	(0.899, 2.330)	4.42	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	0.217	0.439	(-0.644, 1.077)	0.49	0.622	1.44
Block3	0.199	0.441	(-0.664, 1.063)	0.45	0.651	1.44
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
TDZ-K 25 µM	-0.146	0.352	(-0.836, 0.544)	-0.42	0.678	1.00

Regression Equation

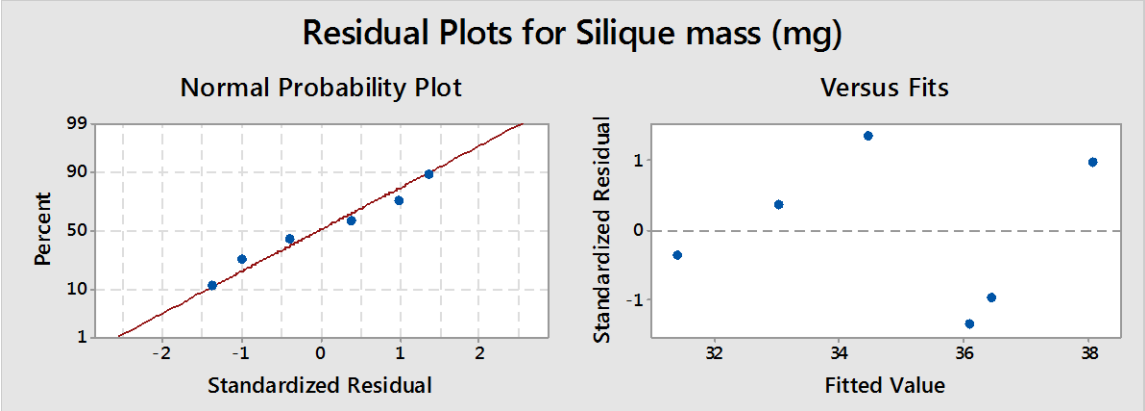
Silique no./plant = exp(Y')

Y' = 1.614 + 0.0Block_Block1 + 0.217Block_Block2 + 0.199Block_Block3
+ 0.0Treatment_Control - 0.146Treatment_TDZ-K 25 µM

Goodness-o*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	2	0.08406	0.04203	0.08	0.959
Pearson	2	0.08399	0.04199	0.08	0.959

2.4.6 Single application of TDZ-K: Silique mass



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	26.113	13.057	1.64	0.378
Treatment	1	3.964	3.964	0.50	0.553
Error	2	15.889	7.944		
Total	5	45.966			

2.4.7 Single application of TDZ-K: Seeds per silique

Poisson Regression Analysis: Seeds/silique versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	0.03721	7.02%	0.03721	0.012403	0.04	0.998
Block	2	0.02000	3.77%	0.02000	0.009998	0.02	0.990
Treatment	1	0.01721	3.25%	0.01721	0.017213	0.02	0.896
Error	2	0.49255	92.98%	0.49255	0.246277		
Total	5	0.52976	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	2.425	0.241	(1.952, 2.898)	10.05	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	0.019	0.294	(-0.557, 0.594)	0.06	0.949	1.35
Block3	0.041	0.292	(-0.531, 0.614)	0.14	0.888	1.35
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
TDZ-K 25 µM	0.031	0.239	(-0.436, 0.499)	0.13	0.896	1.00

Regression Equation

Seeds/silique = exp(Y')

Y' = 2.425 + 0.0Block_Block1 + 0.019Block_Block2 + 0.041Block_Block3
+ 0.0Treatment_Control + 0.031Treatment_TDZ-K 25 µM

Goodness-o*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	2	0.49255	0.24628	0.49	0.782
Pearson	2	0.49136	0.24568	0.49	0.782

2.4.8 Single application of TDZ-K: Seeds per plant

Poisson Regression Analysis: Seeds/plant versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	3	8.609	82.07%	8.609	2.8698	8.61	0.035
Block	2	7.231	68.93%	7.231	3.6154	7.23	0.027
Treatment	1	1.379	13.14%	1.379	1.3785	1.38	0.240
Error	2	1.881	17.93%	1.881	0.9407		
Total	5	10.491	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	3.951	0.112	(3.731, 4.171)	35.16	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	0.301	0.133	(0.039, 0.562)	2.25	0.024	1.50
Block3	0.319	0.133	(0.059, 0.580)	2.41	0.016	1.50
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
TDZ-K 25 µM	-0.123	0.105	(-0.329, 0.083)	-1.17	0.241	1.00

Regression Equation

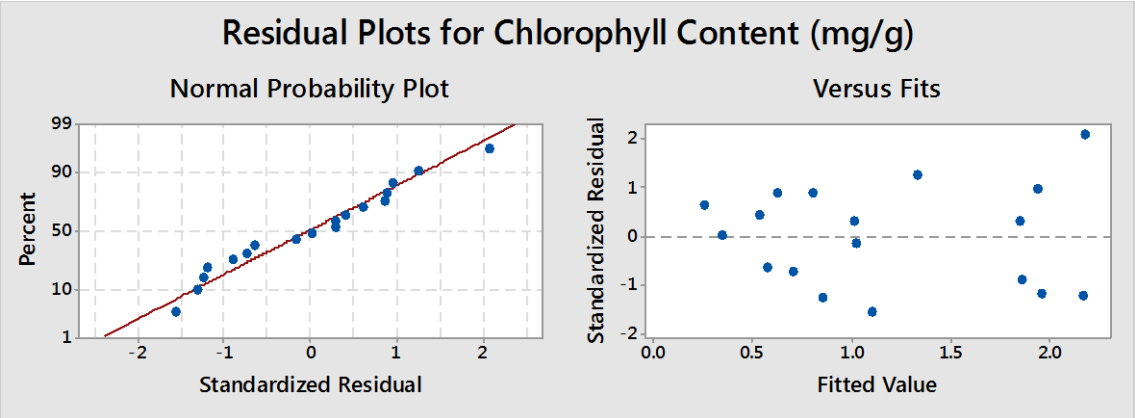
Seeds/plant = exp(Y')

Y' = 3.951 + 0.0Block_Block1 + 0.301Block_Block2 + 0.319Block_Block3
+ 0.0Treatment_Control - 0.123Treatment_TDZ-K 25 µM

Deviance
R-Sq
82.07%

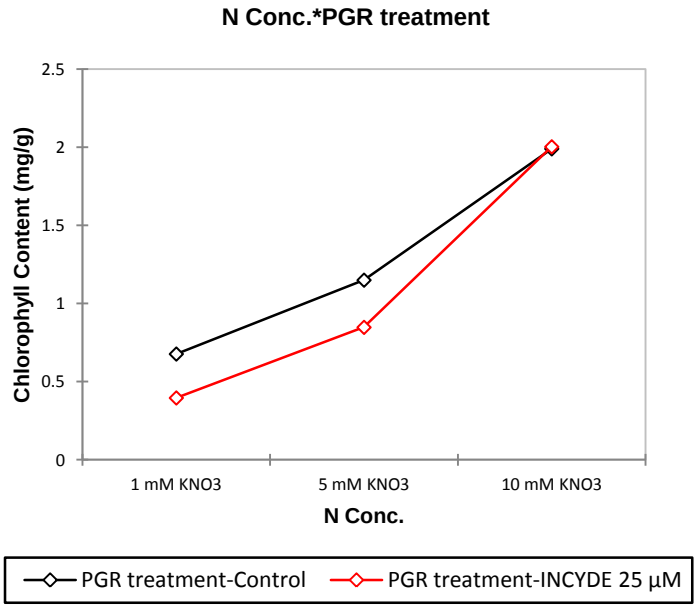
2.5 Chlorophyll content

2.5.1 Chlorophyll top leaf



Analysis of Variance

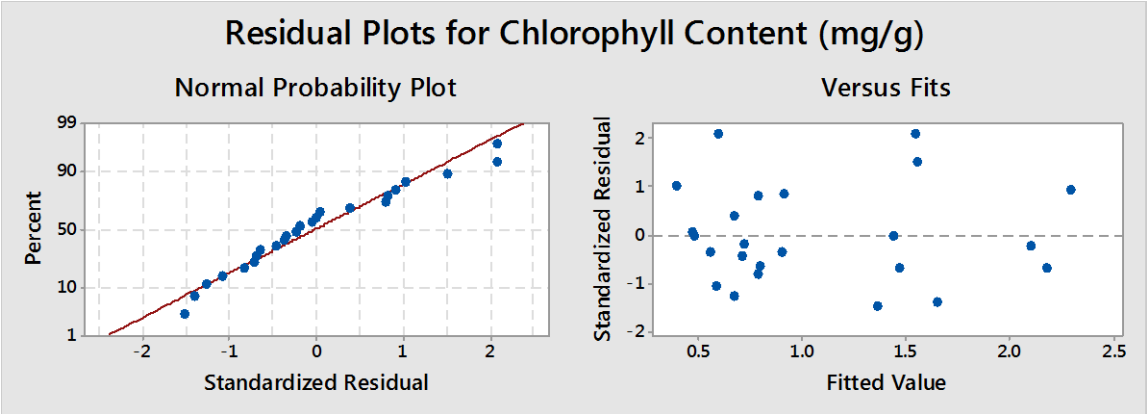
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.3203	0.16014	0.57	0.581
N Conc.	2	6.6770	3.33852	11.94	0.002
PGR treatment	1	0.1623	0.16230	0.58	0.464
N Conc.*PGR treatment	2	0.0929	0.04643	0.17	0.849
Error	10	2.7951	0.27951		
Total	17	10.0476			



N Conc. / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll Content (mg/g)):						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
10 mM KNO3 vs 1 mM KNO3	1.459	4.781	2.741	**0.002**	Yes	
10 mM KNO3 vs 5 mM KNO3	0.998	3.268	2.741	**0.021**	Yes	
5 mM KNO3 vs 1 mM KNO3	0.462	1.513	2.741	0.326	No	
Tukey's d critical value:			3.877			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
10 mM KNO3	1.996	0.216	1.515	2.477	A	
5 mM KNO3	0.999	0.216	0.518	1.480		B
1 mM KNO3	0.537	0.216	0.056	1.018		B
PGR treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll Content (mg/g)):						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
Control vs INCYDE 25 µM	0.190	0.762	2.228	0.464	No	
Tukey's d critical value:			3.151			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
Control	1.272	0.176	0.880	1.665	A	
INCYDE 25 µM	1.082	0.176	0.690	1.475	A	
N Conc.*PGR treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll Content (mg/g)):						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
N Conc.-10 mM KNO3*PGR treatment-INCYDE 25 µM vs N Conc.-10 mM KNO3*PGR treatment-Control	0.013	0.030	3.473	1.000	No	
N Conc.-5 mM KNO3*PGR treatment-Control vs N Conc.-5 mM KNO3*PGR treatment-INCYDE 25 µM	0.302	0.699	3.473	0.978	No	
N Conc.-1 mM KNO3*PGR treatment-Control vs N Conc.-1 mM KNO3*PGR treatment-INCYDE 25 µM	0.281	0.651	3.473	0.984	No	
Tukey's d critical value:			4.912			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
N Conc.-10 mM KNO3*PGR treatment-INCYDE 25 µM	2.003	0.305	1.323	2.683	A	
N Conc.-10 mM KNO3*PGR treatment-Control	1.990	0.305	1.310	2.670	A	
N Conc.-5 mM KNO3*PGR treatment-Control	1.150	0.305	0.470	1.830	A	B
N Conc.-5 mM KNO3*PGR treatment-INCYDE 25 µM	0.848	0.305	0.168	1.528	A	B
N Conc.-1 mM KNO3*PGR treatment-Control	0.677	0.305	-0.003	1.357	A	B
N Conc.-1 mM KNO3*PGR treatment-INCYDE 25 µM	0.396	0.305	-0.284	1.077		B
Summary of all pairwise comparisons for Blocks (Tukey (HSD)):						

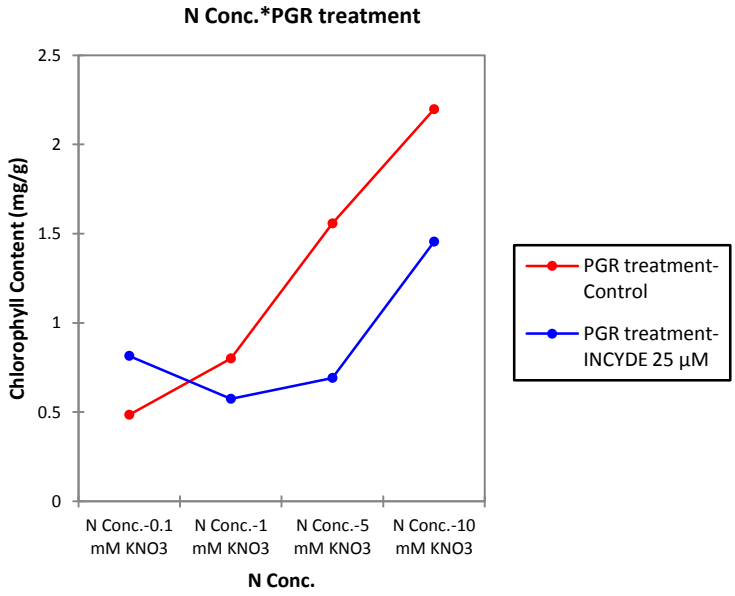
Category	LS means(Chlorophyll Content (mg/g))	Groups				
Block1	1.358	A				
Block3	1.133	A				
Block2	1.041	A				
Summary of all pairwise comparisons for N Conc. (Tukey (HSD)):						
Category	LS means(Chlorophyll Content (mg/g))	Groups				
10 mM KNO3	1.996	A				
5 mM KNO3	0.999		B			
1 mM KNO3	0.537		B			
Summary of all pairwise comparisons for PGR treatment (Tukey (HSD)):						
Category	LS means(Chlorophyll Content (mg/g))	Groups				
Control	1.272	A				
INCYDE 25 µM	1.082	A				
Summary of all pairwise comparisons for N Conc.*PGR treatment (Tukey (HSD)):						
Category	LS means(Chlorophyll Content (mg/g))	Groups				
10 mM KNO3*INCYDE 25 µM	2.003	A				
10 mM KNO3*Control	1.990	A				
5 mM KNO3*Control	1.150	A	B			
5 mM KNO3*INCYDE 25 µM	0.848	A	B			
1 mM KNO3*Control	0.677	A	B			
1 mM KNO3*INCYDE 25 µM	0.396		B			

2.5.2 Chlorophyll bottom leaf



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.1459	0.07296	0.41	0.669
N Conc.	3	5.3852	1.79506	10.17	**0.001**
PGR treatment	1	0.8486	0.84859	4.81	**0.046**
N Conc.*PGR treatment	3	1.3419	0.44729	2.53	0.099
Error	14	2.4718	0.17656		
Total	23	10.1933			



N Conc. / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
10 mM KNO3 vs 0.1 mM KNO3	1.176	4.849	2.907	0.001	Yes	
10 mM KNO3 vs 1 mM KNO3	1.139	4.694	2.907	0.002	Yes	
10 mM KNO3 vs 5 mM KNO3	0.702	2.893	2.907	0.051	No	
5 mM KNO3 vs 0.1 mM KNO3	0.474	1.955	2.907	0.251	No	
5 mM KNO3 vs 1 mM KNO3	0.437	1.801	2.907	0.314	No	
1 mM KNO3 vs 0.1 mM KNO3	0.038	0.155	2.907	0.999	No	
Tukey's d critical value:			4.111			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
10 mM KNO3	1.826	0.172	1.458	2.194	A	
5 mM KNO3	1.124	0.172	0.756	1.492	A	B
1 mM KNO3	0.687	0.172	0.320	1.055		B
0.1 mM KNO3	0.650	0.172	0.282	1.018		B
PGR treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
Control vs INCYDE 25 µM	0.376	2.192	2.145	0.046	Yes	
Tukey's d critical value:			3.033			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
Control	1.260	0.121	1.000	1.520	A	
INCYDE 25 µM	0.884	0.121	0.624	1.144		B
N Conc.*PGR treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
10 mM KNO3*Control vs 10 mM KNO3*INCYDE 25 µM	0.742	2.163	3.529	0.425	No	
5 mM KNO3*Control vs 5 mM KNO3*INCYDE 25 µM	0.866	2.524	3.529	0.261	No	
0.1 mM KNO3*INCYDE 25 µM vs 0.1 mM KNO3*Control	0.330	0.962	3.529	0.973	No	
1 mM KNO3*Control vs 1 mM KNO3*INCYDE 25 µM	0.226	0.660	3.529	0.997	No	
Tukey's d critical value:			4.99			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
10 mM KNO3*Control	2.197	0.243	1.677	2.718	A	
5 mM KNO3*Control	1.557	0.243	1.037	2.077	A	B
10 mM KNO3*INCYDE 25 µM	1.455	0.243	0.935	1.975	A	B
0.1 mM KNO3*INCYDE 25 µM	0.815	0.243	0.295	1.335		B
1 mM KNO3*Control	0.801	0.243	0.280	1.321		B
5 mM KNO3*INCYDE 25 µM	0.691	0.243	0.171	1.212		B
1 mM KNO3*INCYDE 25 µM	0.574	0.243	0.054	1.095		B
0.1 mM KNO3*Control	0.485	0.243	-0.035	1.005		B

Appendix 3: Chapter 3 statistical analyses for field trials with wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.)

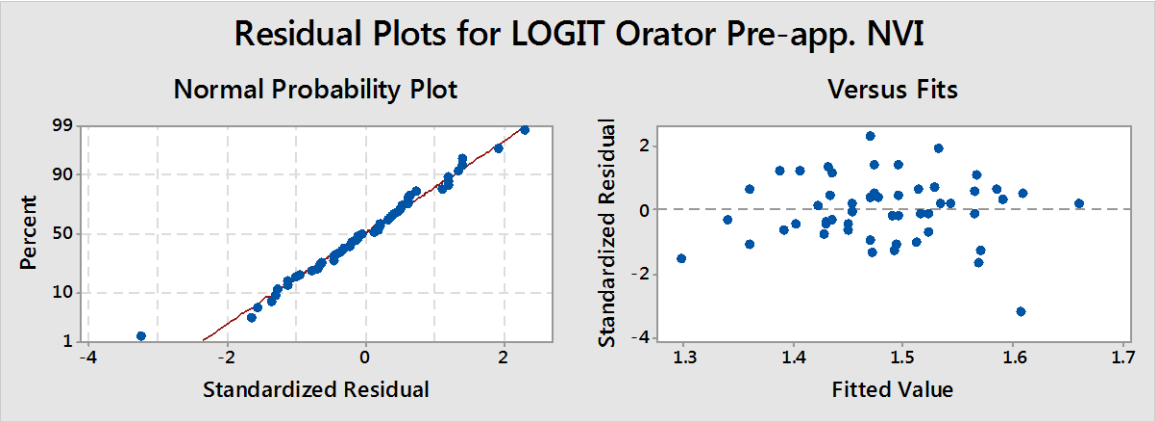
3.1 Summary of application rates for field trials

	Wheat cv. Orator (2013-2014 trial)	**Wheat cv. Torch (2014-2015 trial)**	**Barley cv. Quench (2014-2015 trial)**
Sowing date (dd/mm/yy) and rate.	29.04.13 cv. Orator 90 kg/ha	16.05.14 cv. Torch 100 kg/ha	10.09.14 cv. Quench 115 kg/ha
Base fertiliser app.	Sulphur super / Pot. chloride mix - 400 kg/ha	Sulphur super / Pot. chloride mix - 400 kg/ha	Crop 20 250 kg/ha at sowing
Spring fertiliser app.	Ammo 36 190 kg/ha Urea 225 kg/ha SustaiN 200 kg/ha SustaiN 150 kg/ha	Ammo 36 200 kg/ha SustaiN 150 kg/ha SustaiN 150 kg/ha	SustaiN 150 kg/ha
Herbicide and insecticide app.	Firebird 0.3 L + Protugan 1.0 L, Twinax 330 mL + Adigor 1.0 L + Karate Zeon 40 mL, Tricombi 3.75 L, Twinax 350 mL + Adigor 1.0 L	Firebird 0.3 L + Protugen 1.0 L, Karate Zeon 30 mL, Transform 75 mL, Twinax 0.3 L + Adigor 1.0 L	Maestro 2.0 L + Quantum 0.2 L
Plant growth regulator	26.09.13 – CCC 1.75 L + Moddus 0.2 L	08.10.14 – CCC 1.5L + Moddus 0.4L	None
Fungicide	Stellar 0.7 Stellar 0.5 L + Comet 0.4 L Stellar 0.75 L + Comet 0.5 L	Stellar 1.0 L + Amistar 0.5 L Stellar 1.0 L + Amistar 0.5 L	Proline 0.4 L + Acanto 0.25 L Seguris Flexi 0.5 L + Proline 0.4 L
Treatment date	GS39 (1.11.13), GS51 (18.11.13), GS61 (29.11.13), GS65 (3.12.13), GS65+13d (16.12.13)	GS51 (21.11.14), GS61 (27.11.14), GS65 (2.12.14), GS65+15d (17.12.14)	GS39 (12.11.14), GS51 (27.11.14), GS61 (11.12.14), GS65 (17.12.14)
Irrigation rate	4 x 40 mm passes (160 mm total)	20.10.14 – 35 mm 13.11.14 – 35 mm 23.11.14 – 35 mm 09.12.14 – 40 mm 01.01.15 – 40 mm 17.01.15 – 40 mm (Total 225 mm)	24.10.14 – 40 mm 04.11.14 – 40 mm 20.11.14 – 40 mm 30.11.14 – 40 mm 14.12.14 – 40 mm 01.01.15 – 40 mm 12.01.15 – 40 mm (Total 280 mm)
Harvest date	12.2.14	16.2.15	13.2.15

3.2 NDVI data

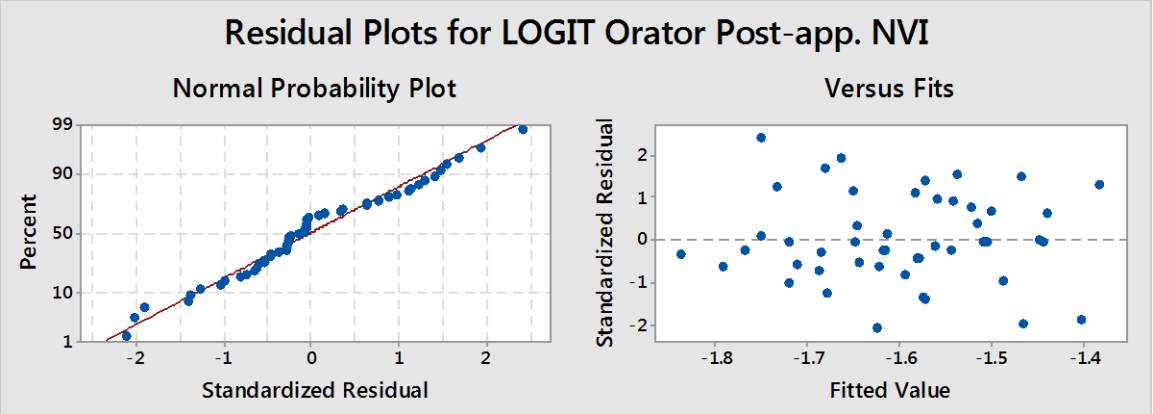
3.2.1 Orator wheat 2013/14: NDVI

	NDVI	
Treatment	Pre-application	Post-application
Nil	0.81 ± 0.005	0.16 ± 0.017
INCYDE 10 µM (GS 65)	0.81 ± 0.007	0.20 ± 0.019
INCYDE 25 µM (GS 39, 51, 61, 65)	0.82 ± 0.005	0.15 ± 0.011
INCYDE 25 µM (GS 39)	0.82 ± 0.008	0.17 ± 0.009
INCYDE 25 µM (GS 51)	0.82 ± 0.008	0.17 ± 0.013
INCYDE 25 µM (GS 61)	0.81 ± 0.004	0.18 ± 0.018
INCYDE 25 µM (GS 65)	0.79 ± 0.011	0.16 ± 0.017
INCYDE 50 µM (GS 61)	0.83 ± 0.006	0.17 ± 0.011
INCYDE 50 µM (GS 65)	0.82 ± 0.010	0.19 ± 0.018
DMSO Control (GS 39, 51, 61, 65)	0.82 ± 0.002	0.16 ± 0.009
TDZ-K 10 µM (GS 61, 65, 65+13d)	0.80 ± 0.010	0.16 ± 0.019
TDZ-K 25 µM (GS 61, 65, 65+13d)	0.82 ± 0.005	0.18 ± 0.009
DMSO Control (GS 61, 65, 65+13d)	0.82 ± 0.007	0.19 ± 0.016
F-value	1.88	0.82
p-value	0.071	0.628



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	0.1284	0.042784	6.30	0.002
Treatment	12	0.1532	0.012768	1.88	0.071
Error	36	0.2443	0.006786		
Total	51	0.5259			

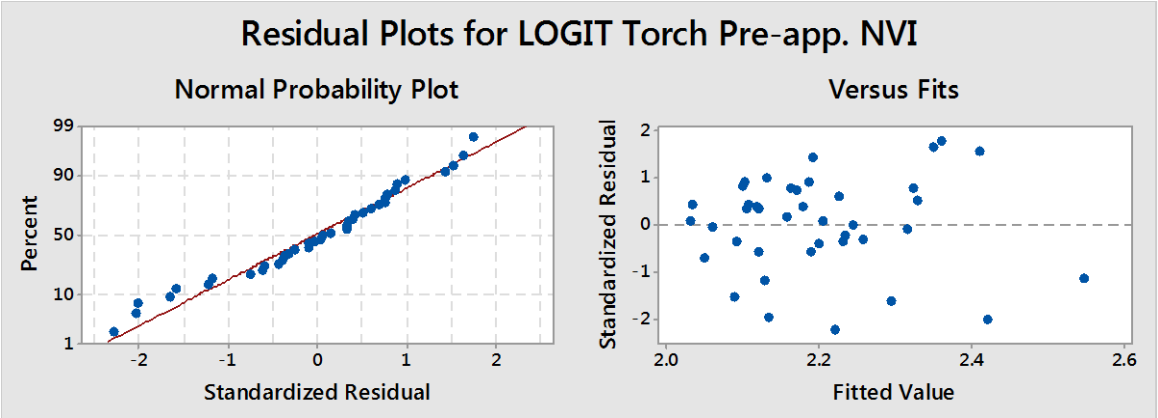


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	0.1220	0.04068	0.92	0.439
Treatment	12	0.4343	0.03619	0.82	0.628
Error	36	1.5857	0.04405		
Total	51	2.1419			

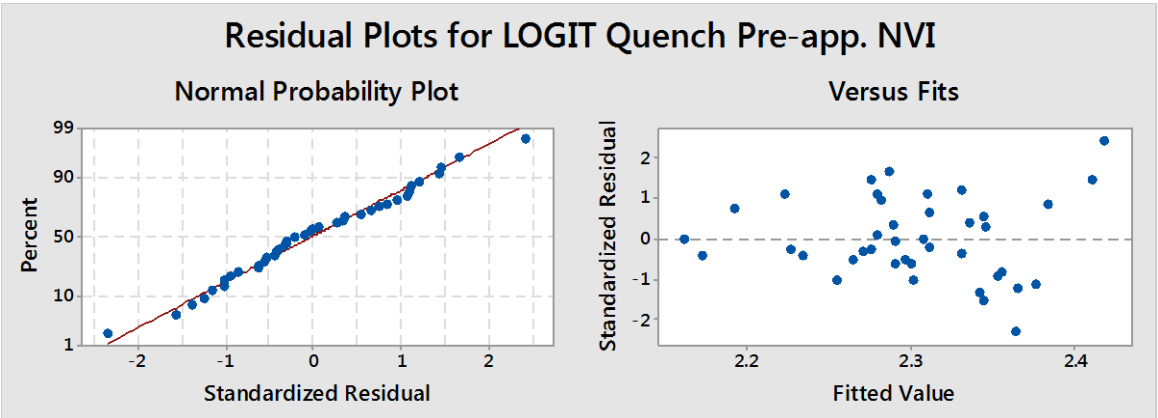
3.2.2 Torch wheat and Quench barley 2014/15: NDVI

Torch wheat 2014/15		Quench barley 2014/15	
Treatment	Pre-application NDVI	Treatment	Pre-application NDVI
Nil	0.89 ± 0.003	Nil	0.91 ± 0.002
DMSO Control (GS 51, 61, 65, 65+15d)	0.92 ± 0.008	DMSO Control (GS 51, 61, 65, 65+15d)	0.91 ± 0.003
TDZ-K 10 µM (GS 61, 65, 65+15d)	0.90 ± 0.012	INC 10 µM (GS 65)	0.90 ± 0.003
TDZ-K 50 µM (GS 61, 65, 65+15d)	0.90 ± 0.004	INC 25 µM (GS 39, 51, 61, 65)	0.91 ± 0.003
CPPU 10 µM (GS 61, 65)	0.89 ± 0.005	INC 25 µM (GS 39)	0.91 ± 0.006
CPPU 30 µM (GS 61, 65)	0.89 ± 0.006	INC 25 µM (GS 51)	0.91 ± 0.003
CPPU 100 µM (GS 61, 65)	0.90 ± 0.012	INC 25 µM (GS 61)	0.90 ± 0.002
CPPU 10 µM (GS 51, 65)	0.89 ± 0.010	INC 25 µM (GS 65)	0.91 ± 0.003
CPPU 30 µM (GS 51, 65)	0.90 ± 0.004	INC 50 µM (GS 61)	0.91 ± 0.002
CPPU 100 µM (GS 51, 65)	0.90 ± 0.009	INC 50 µM (GS 65)	0.91 ± 0.004
F-value	1.3	*F*-value	2.09
p-value	0.28	*p*-value	0.067



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	0.2050	0.06832	2.50	0.080
Treatment	9	0.3202	0.03558	1.30	0.280
Error	27	0.7367	0.02728		
Total	39	1.2618			

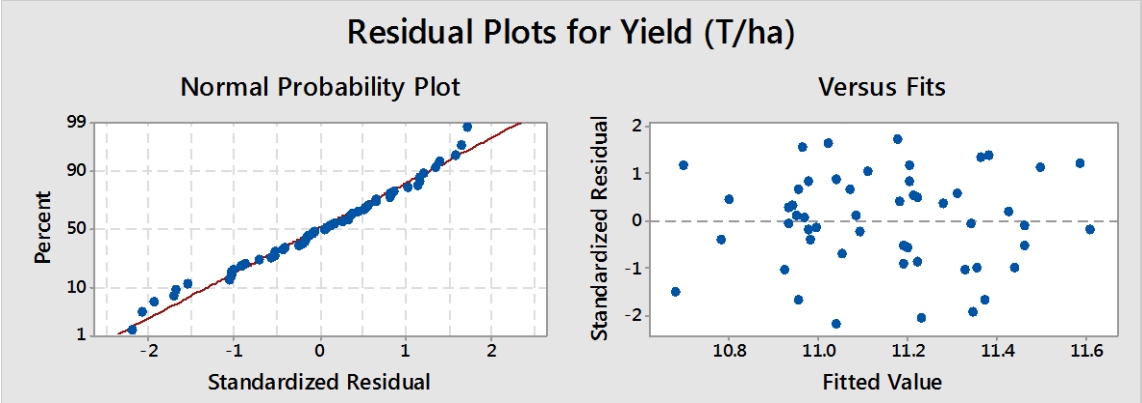


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	0.02497	0.008323	1.44	0.252
Treatment	9	0.10874	0.012082	2.09	0.067
Error	27	0.15584	0.005772		
Total	39	0.28955			

3.3 Grain lab results

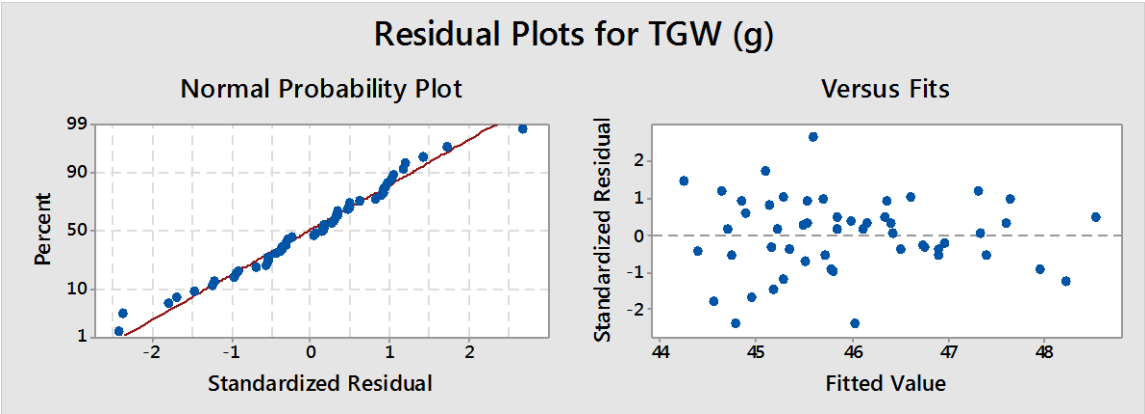
3.3.1 Orator wheat 2013/14: Yield (T/ha)



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	1.5004	0.50015	11.40	0.000
Treatment	12	0.9967	0.08306	1.89	0.069
Error	36	1.5790	0.04386		
Total	51	4.0761			

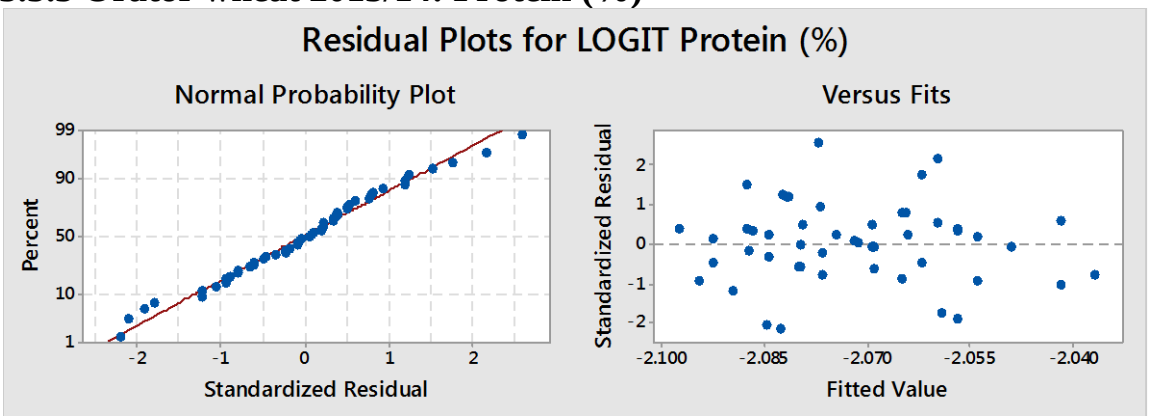
3.3.2 Orator wheat 2013/14: TGW (g)



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	35.08	11.693	6.08	0.002
Treatment	12	20.43	1.703	0.89	0.569
Error	36	69.25	1.924		
Total	51	124.76			

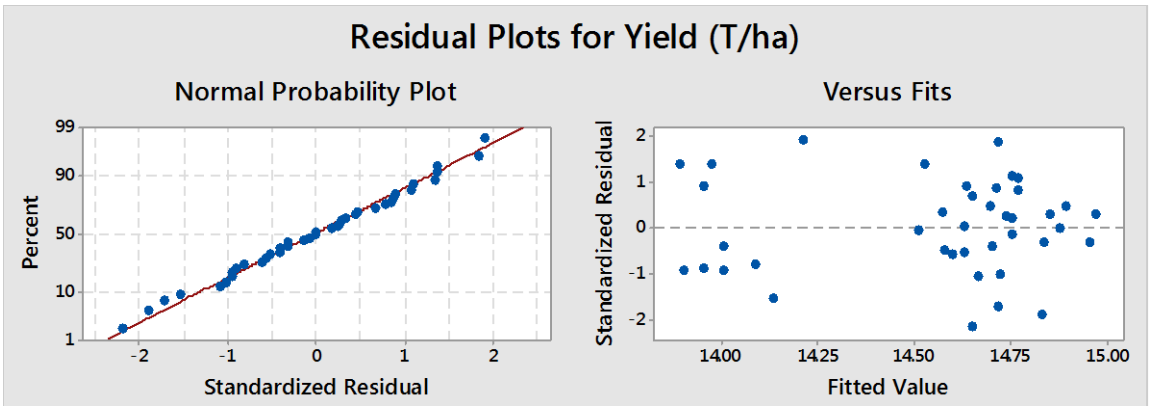
3.3.3 Orator wheat 2013/14: Protein (%)



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	0.004200	0.001400	3.09	0.039
Treatment	12	0.006562	0.000547	1.21	0.316
Error	36	0.016317	0.000453		
Total	51	0.027079			

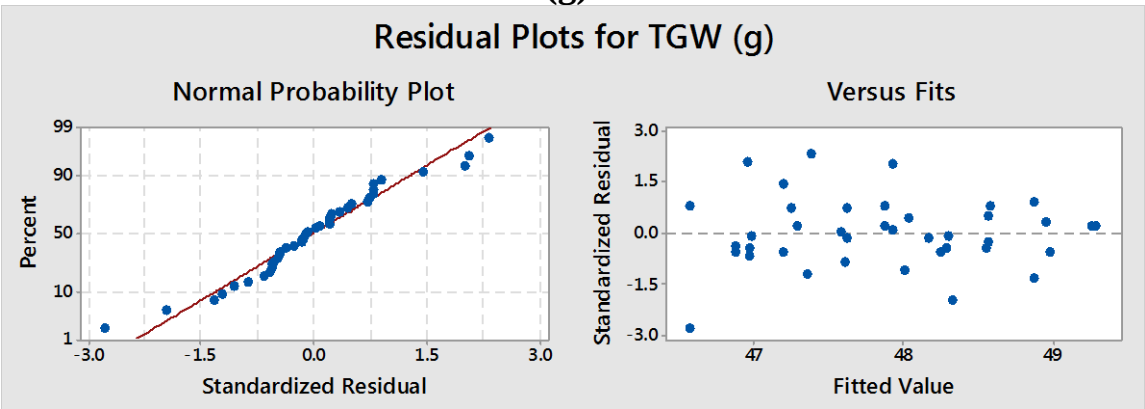
3.3.4 Torch wheat 2014/15 : Yield (T/ha)



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	3.9088	1.30295	14.15	0.000
Treatment	9	0.3775	0.04194	0.46	0.891
Error	27	2.4857	0.09206		
Total	39	6.7720			

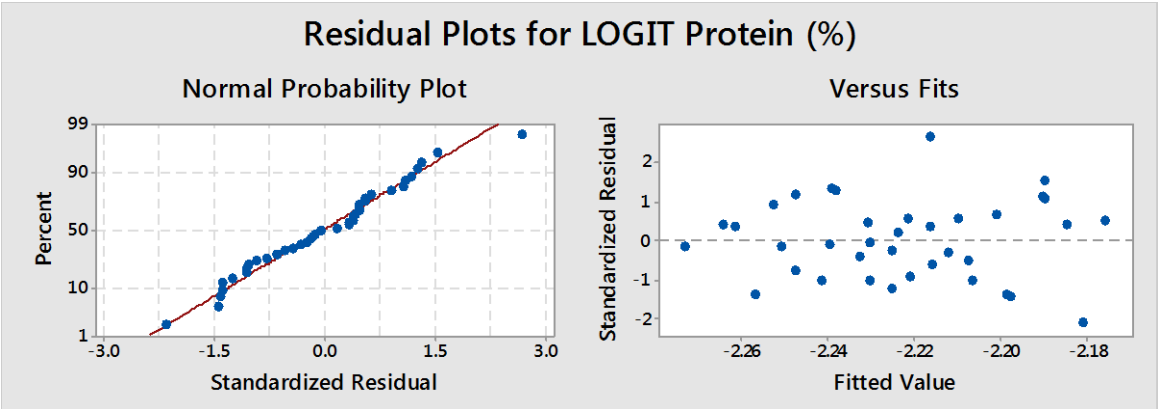
3.3.5 Torch wheat 2014/15 : TGW (g)



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	1.565	0.5216	0.33	0.803
Treatment	9	20.680	2.2978	1.46	0.214
Error	27	42.543	1.5757		
Total	39	64.788			

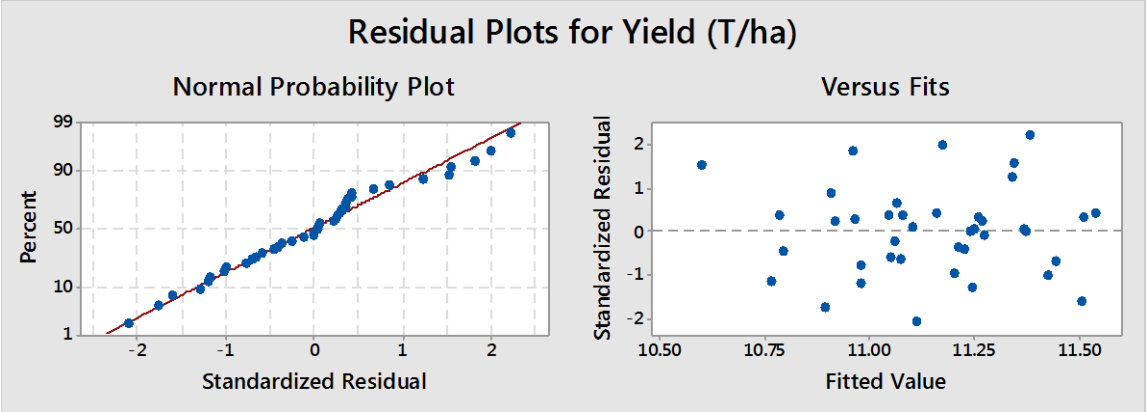
3.3.6 Torch wheat 2014/15 : Protein (%)



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	0.017274	0.005758	7.17	0.001
Treatment	9	0.009214	0.001024	1.27	0.295
Error	27	0.021687	0.000803		
Total	39	0.048175			

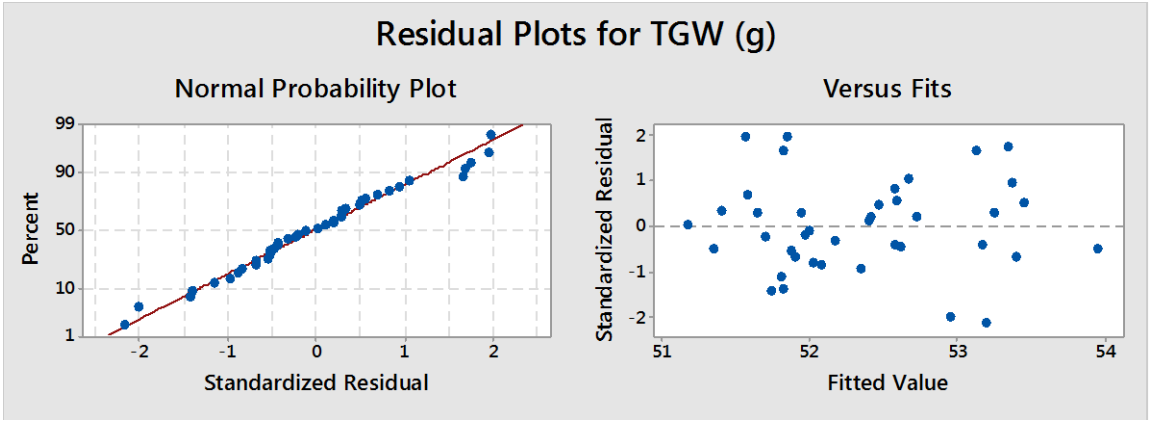
3.3.7 Quench barley 2014/15: Yield (T/ha)



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	1.1282	0.37606	4.58	0.010
Treatment	9	0.8256	0.09173	1.12	0.385
Error	27	2.2180	0.08215		
Total	39	4.1718			

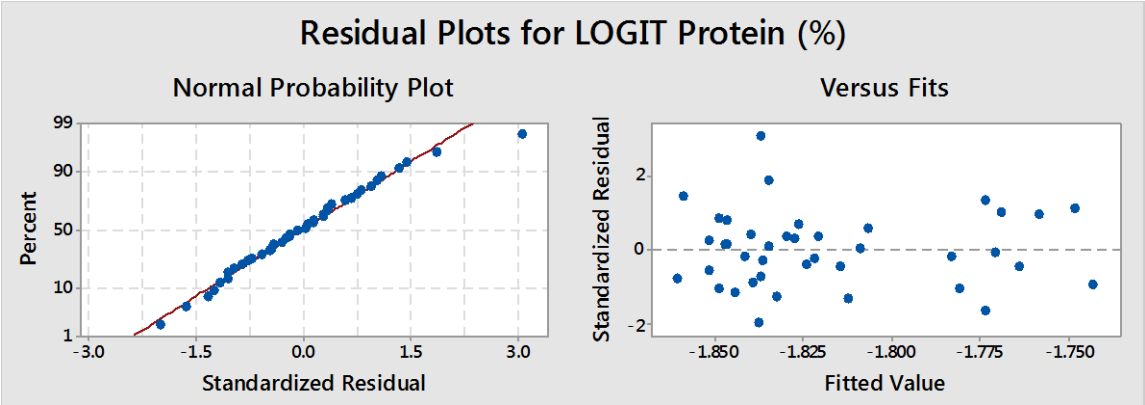
3.3.8 Quench barley 2014/15: TGW (g)



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	14.743	4.9143	4.31	0.013
Treatment	9	3.817	0.4241	0.37	0.938
Error	27	30.780	1.1400		
Total	39	49.340			

3.3.9 Quench barley 2014/15: Protein (%)

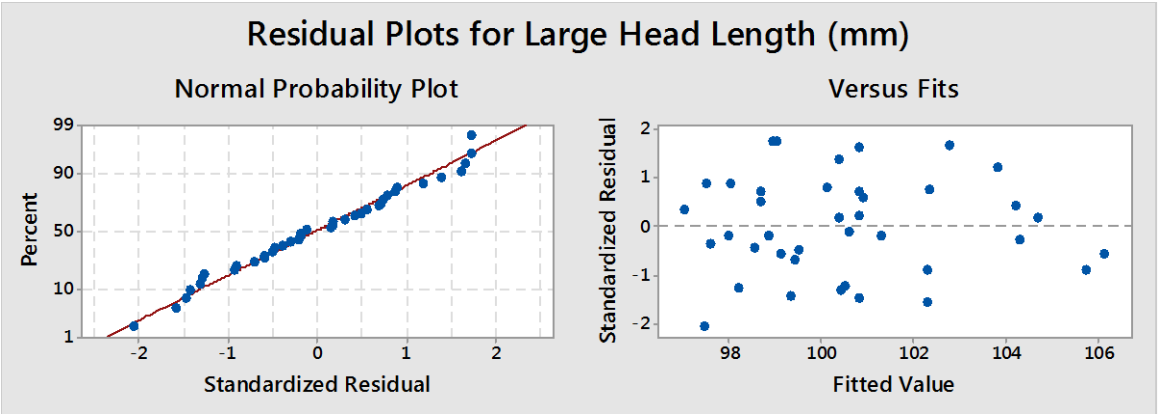


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	0.037130	0.012377	4.98	0.007
Treatment	9	0.006213	0.000690	0.28	0.975
Error	27	0.067127	0.002486		
Total	39	0.110470			

3.4 Orator wheat 2013/14

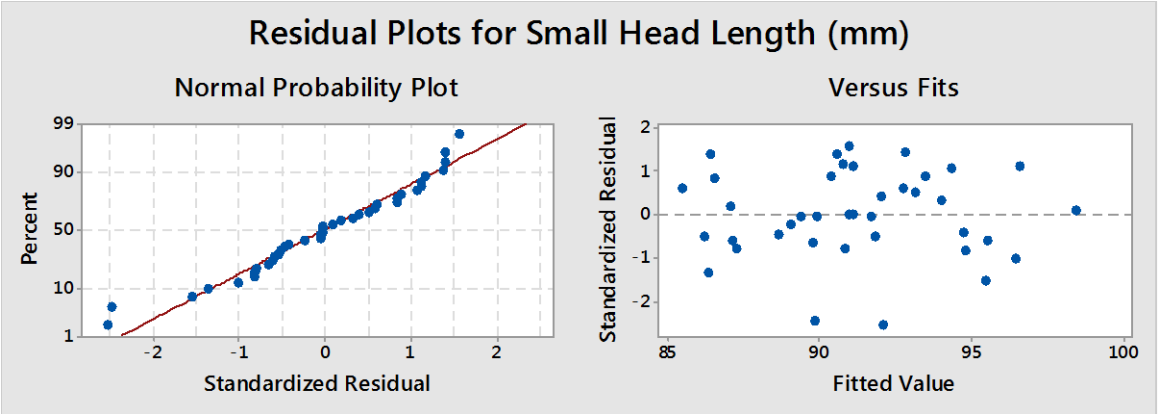
3.4.1 Orator wheat 2013/14: Large Head Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	26.59	13.296	1.34	0.280
Treatment	12	186.95	15.579	1.57	0.167
Error	24	237.86	9.911		
Total	38	451.39			

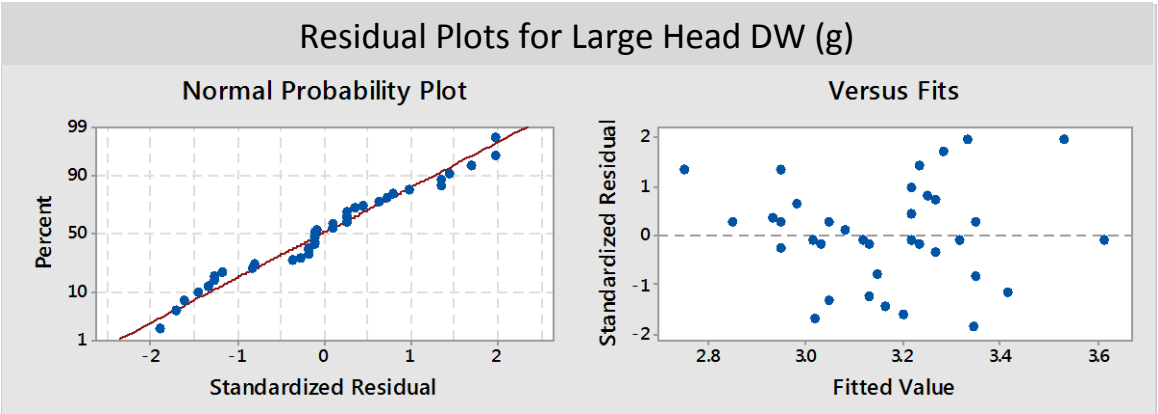
3.4.2 Orator wheat 2013/14: Small Head Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	150.0	74.99	2.54	0.100
Treatment	12	254.7	21.22	0.72	0.719
Error	24	707.9	29.50		
Total	38	1112.6			

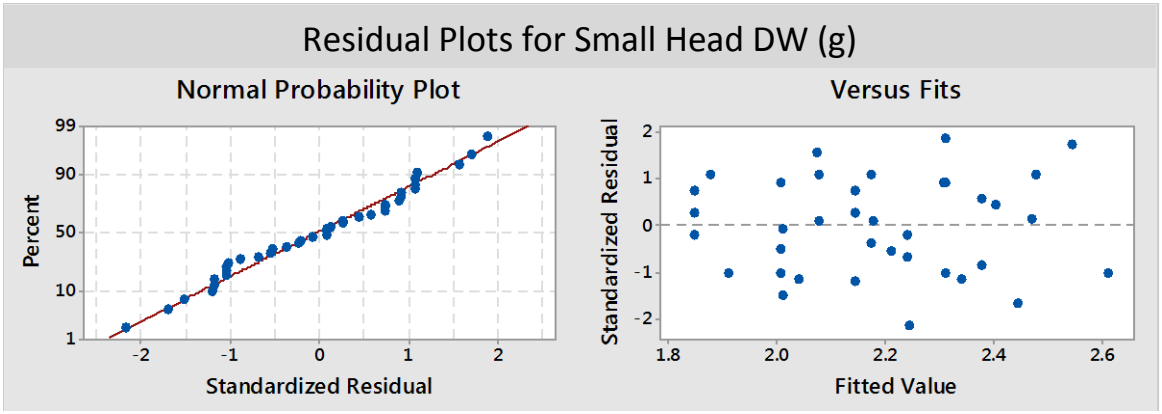
3.4.3 Orator wheat 2013/14: Large Head DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.4928	0.24641	4.43	0.023
Treatment	12	0.7600	0.06333	1.14	0.376
Error	24	1.3338	0.05558		
Total	38	2.5867			

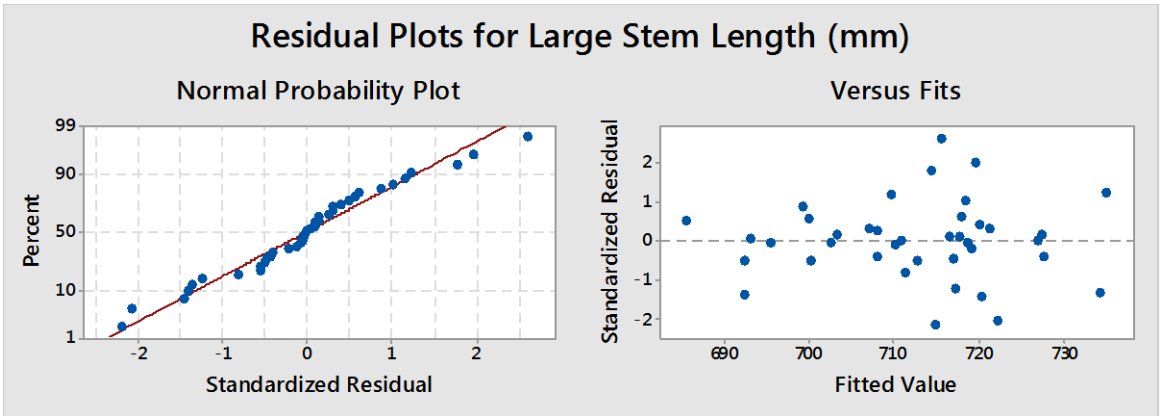
3.4.4 Orator wheat 2013/14: Small Head DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.5862	0.29308	4.27	0.026
Treatment	12	0.9174	0.07645	1.11	0.394
Error	24	1.6472	0.06863		
Total	38	3.1508			

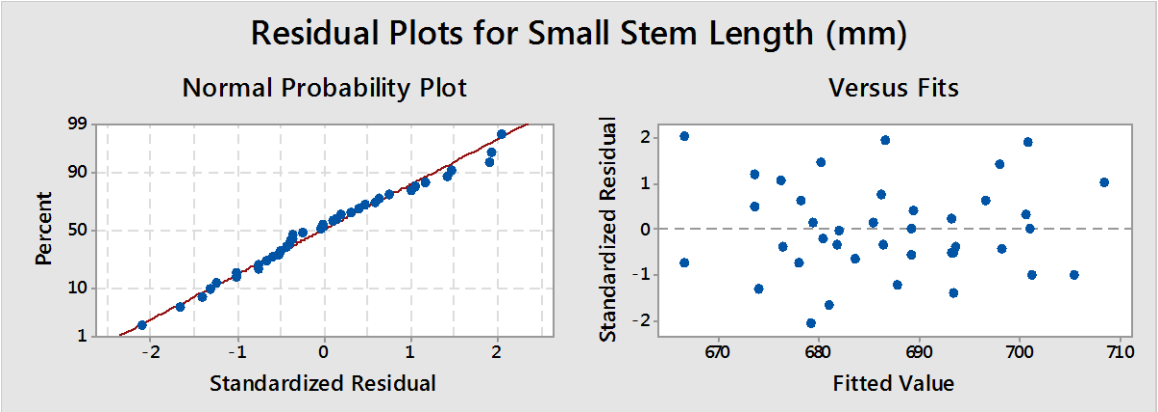
3.4.5Orator wheat 2013/14: Large Stem Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	469.3	234.7	0.66	0.526
Treatment	12	4721.3	393.4	1.11	0.399
Error	24	8533.4	355.6		
Total	38	13724.0			

3.4.6 Orator wheat 2013/14: Small Stem Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	479.7	239.8	0.82	0.453
Treatment	12	3730.5	310.9	1.06	0.432
Error	24	7040.4	293.4		
Total	38	11250.7			

3.4.7 Orator wheat 2013/14: Tiller no.

Poisson Regression Analysis: Tiller no. versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	14	0.61662	42.94%	0.61662	0.04404	0.62	1.000
Block	2	0.07161	4.99%	0.07161	0.03581	0.07	0.965
Treatment	12	0.54501	37.95%	0.54501	0.04542	0.55	1.000
Error	24	0.81947	57.06%	0.81947	0.03414		
Total	38	1.43609	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value
Constant	1.301	0.326	(0.661, 1.941)	3.98
Block				
Block1	0.000000	0.000000	(0.000000, 0.000000)	*
Block2	-0.035	0.202	(-0.430, 0.361)	-0.17
Block3	-0.053	0.203	(-0.451, 0.344)	-0.26
Treatment				
DMSO Control (GS 39, 51, 61, 65)	0.000000	0.000000	(0.000000, 0.000000)	*
DMSO Control (GS 61, 65, 65+13d)	0.055	0.427	(-0.781, 0.891)	0.13
INCYDE 10 µM (GS 65)	0.115	0.420	(-0.709, 0.939)	0.27
INCYDE 25 µM (GS 39)	0.202	0.412	(-0.605, 1.010)	0.49
INCYDE 25 µM (GS 39, 51, 61, 65)	-0.038	0.437	(-0.894, 0.817)	-0.09
INCYDE 25 µM (GS 51)	0.037	0.428	(-0.803, 0.876)	0.09
INCYDE 25 µM (GS 61)	0.072	0.425	(-0.760, 0.905)	0.17
INCYDE 25 µM (GS 65)	0.063	0.426	(-0.771, 0.898)	0.15
INCYDE 50 µM (GS 61)	0.009	0.431	(-0.836, 0.855)	0.02
INCYDE 50 µM (GS 65)	-0.009	0.433	(-0.859, 0.840)	-0.02
Nil	0.046	0.427	(-0.792, 0.884)	0.11
TDZ-K 10 µM (GS 61, 65, 65+13d)	0.028	0.429	(-0.814, 0.869)	0.06
TDZ-K 25 µM (GS 61, 65, 65+13d)	-0.000	0.432	(-0.847, 0.847)	-0.00

Term	*P*-Value	VIF
Constant	0.000	
Block		
Block1	*	*
Block2	0.864	1.31
Block3	0.792	1.31
Treatment		
DMSO Control (GS 39, 51, 61, 65)	*	*
DMSO Control (GS 61, 65, 65+13d)	0.898	1.90
INCYDE 10 µM (GS 65)	0.785	1.95
INCYDE 25 µM (GS 39)	0.623	2.02
INCYDE 25 µM (GS 39, 51, 61, 65)	0.930	1.82
INCYDE 25 µM (GS 51)	0.932	1.88
INCYDE 25 µM (GS 61)	0.865	1.91
INCYDE 25 µM (GS 65)	0.882	1.90
INCYDE 50 µM (GS 61)	0.983	1.86
INCYDE 50 µM (GS 65)	0.983	1.85
Nil	0.915	1.89
TDZ-K 10 µM (GS 61, 65, 65+13d)	0.949	1.87
TDZ-K 25 µM (GS 61, 65, 65+13d)	1.000	1.85

Regression Equation

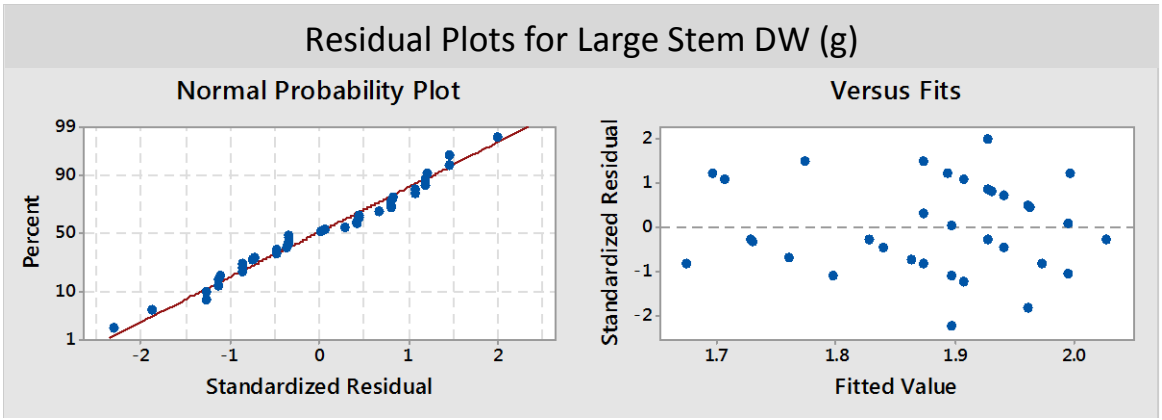
Tiller no. = exp(Y')

Y' = 1.301 +0.0Block_Block1 -0.035Block_Block2 -0.053 Block_Block3
+0.0Treatment_DMSO Control (GS 39, 51, 61, 65) +0.055Treatment_DMSO Control (GS 61, 65, 65+13d) +0.115Treatment_INCYDE 10 µM (GS 65) +0.202Treatment_INCYDE 25 µM (GS 39) -0.038Treatment_INCYDE 25 µM (GS 39, 51, 61, 65) +0.037Treatment_INCYDE 25 µM (GS 51) +0.072Treatment_INCYDE 25 µM (GS 61) +0.063Treatment_INCYDE 25 µM (GS 65) +0.009Treatment_INCYDE 50 µM (GS 61) -0.009Treatment_INCYDE 50 µM (GS 65) +0.046Treatment_Nil +0.028Treatment_TDZ-K 10 µM (GS 61, 65, 65+13d) -0.000Treatment_TDZ-K 25 µM (GS 61, 65, 65+13d)

Goodness-o*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	24	0.81947	0.03414	0.82	1.000
Pearson	24	0.81504	0.03396	0.82	1.000

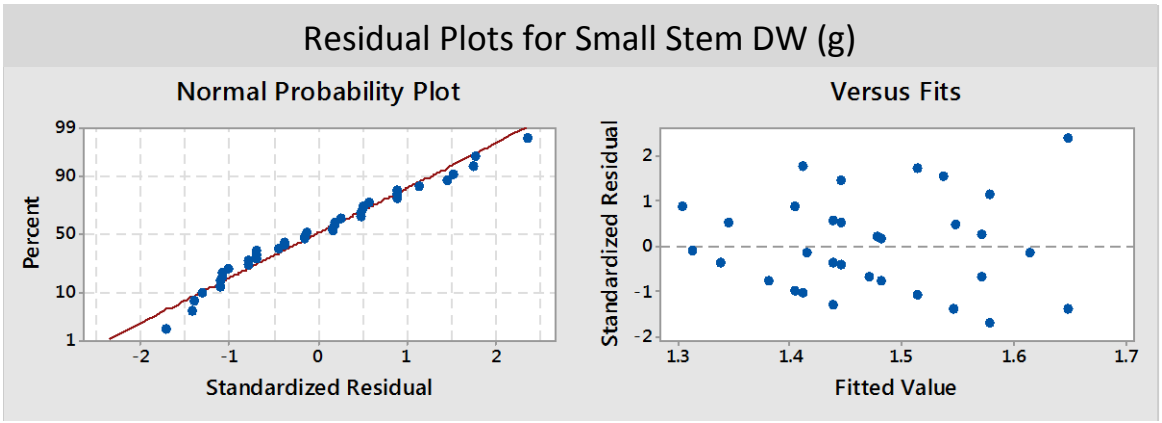
3.4.8 Orator wheat 2013/14: Large Stem DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.01897	0.009487	0.79	0.465
Treatment	12	0.29692	0.024744	2.06	0.063
Error	24	0.28769	0.011987		
Total	38	0.60359			

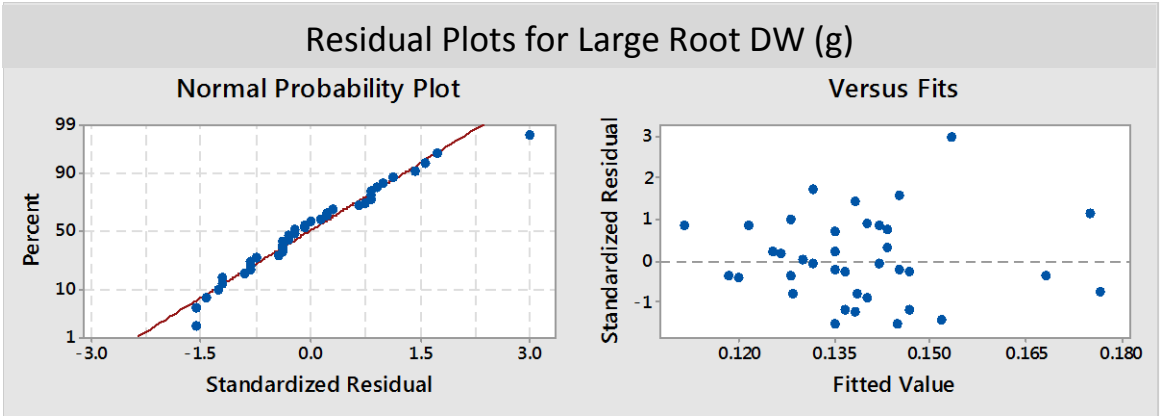
3.4.9 Orator wheat 2013/14: Small Stem DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.04667	0.02333	1.29	0.293
Treatment	12	0.23436	0.01953	1.08	0.416
Error	24	0.43333	0.01806		
Total	38	0.71436			

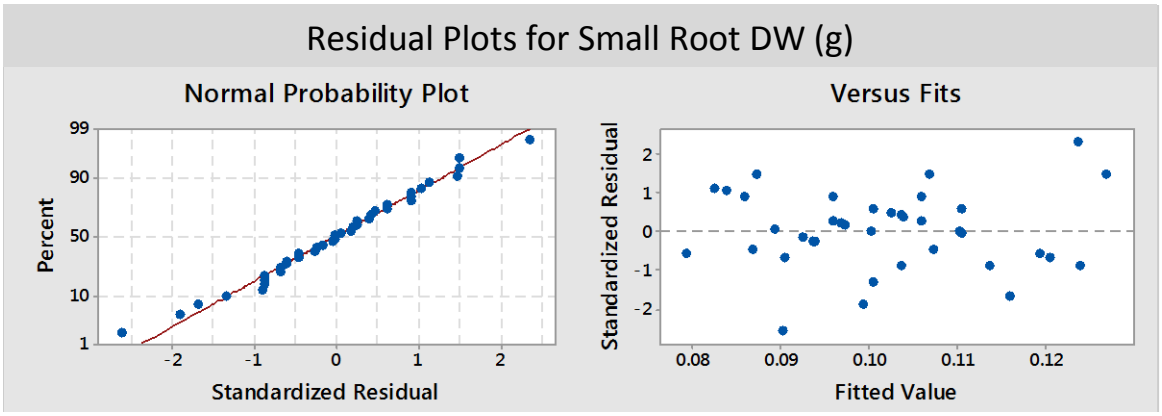
3.4.10 Orator wheat 2013/14: Large Root DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.000528	0.000264	0.33	0.721
Treatment	12	0.006908	0.000576	0.72	0.717
Error	24	0.019138	0.000797		
Total	38	0.026574			

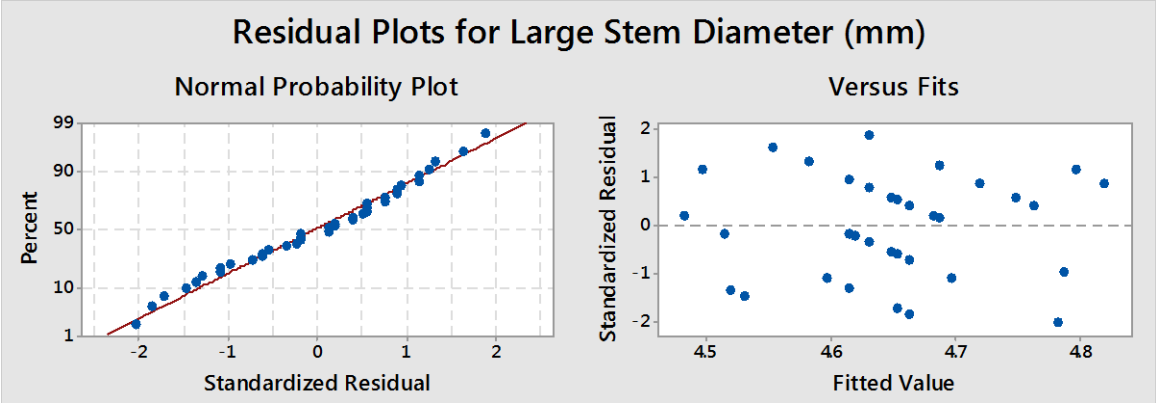
3.4.11 Orator wheat 2013/14: Small Root DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.000390	0.000195	0.50	0.610
Treatment	12	0.005308	0.000442	1.14	0.373
Error	24	0.009277	0.000387		
Total	38	0.014974			

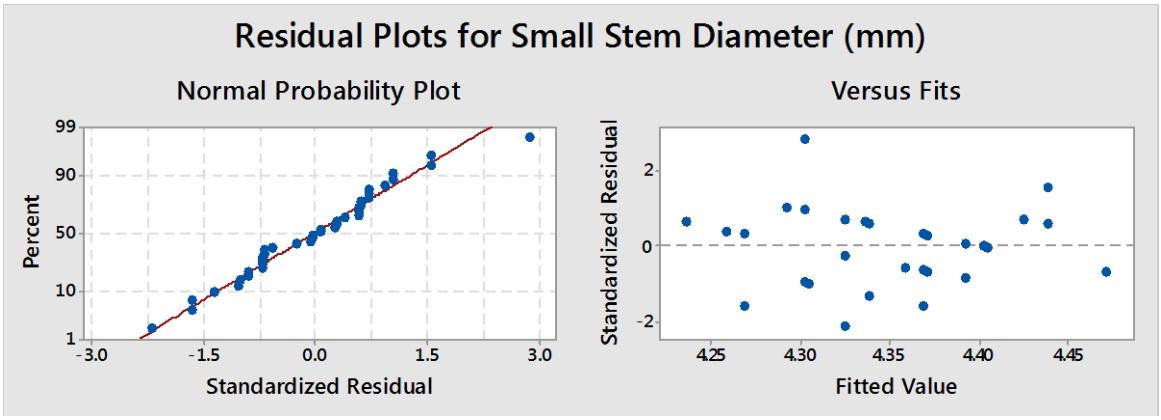
3.4.12 Orator wheat 2013/14: Large Stem Diameter



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.009744	0.004872	0.38	0.690
Treatment	12	0.237436	0.019786	1.53	0.181
Error	24	0.310256	0.012927		
Total	38	0.557436			

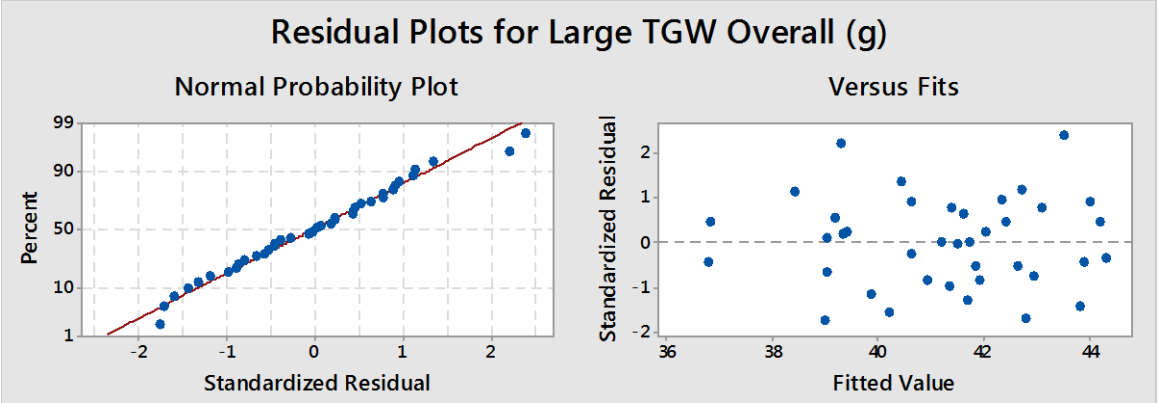
3.4.13 Orator wheat 2013/14: Small Stem Diameter



Analysis of Variance

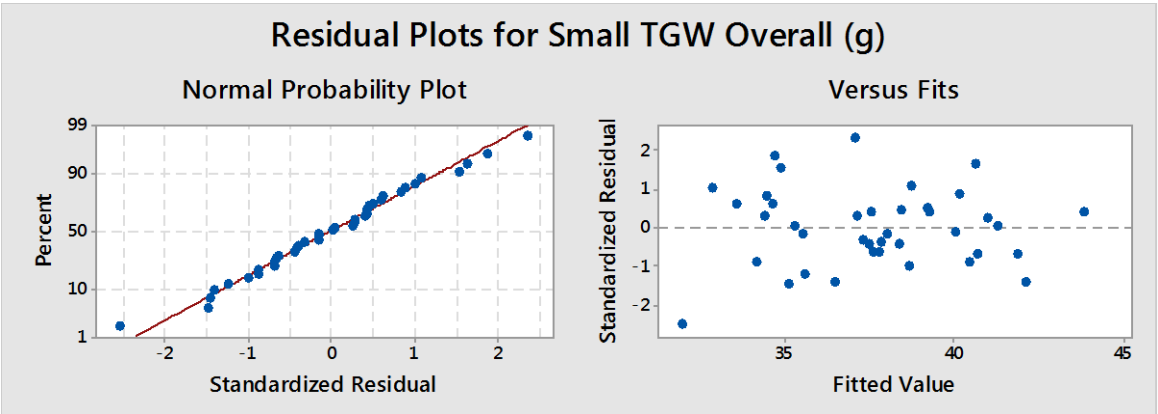
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.03231	0.016154	0.94	0.406
Treatment	12	0.10564	0.008803	0.51	0.888
Error	24	0.41436	0.017265		
Total	38	0.55231			

3.4.14 Orator wheat 2013/14: Large TGW Overall



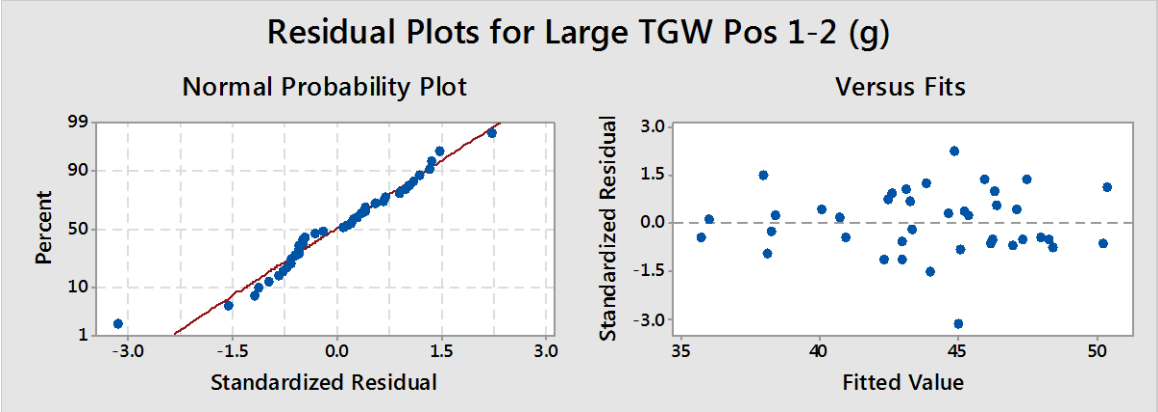
Analysis of Variance					
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	48.01	24.007	2.86	0.077
Treatment	12	96.52	8.043	0.96	0.511
Error	24	201.32	8.388		
Total	38	345.86			

3.4.15 Orator wheat 2013/14: Small TGW Overall



Analysis of Variance					
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	57.17	28.59	1.67	0.209
Treatment	12	243.06	20.25	1.18	0.347
Error	24	410.26	17.09		
Total	38	710.49			

3.4.16 Orator wheat 2013/14: Large TGW Pos 1-2



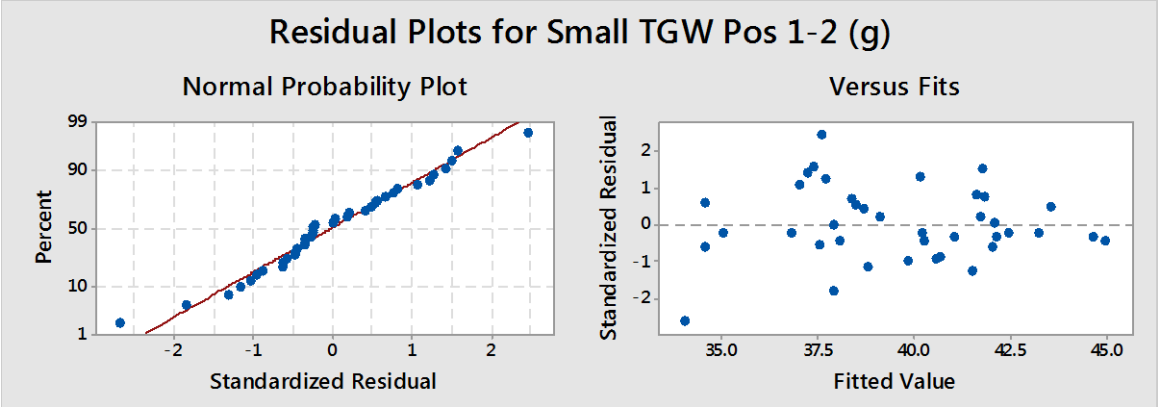
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	48.14	24.07	1.33	0.283
Treatment	12	474.30	39.52	2.19	**0.050**
Error	24	433.73	18.07		
Total	38	956.17			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:					
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
INCYDE 25 µM (GS 39) vs DMSO Control (GS 39, 51, 61, 65)	5.363	1.545	3.662	0.930	No
INCYDE 25 µM (GS 39) vs DMSO Control (GS 61, 65, 65+13d)	5.013	1.444	3.662	0.955	No
INCYDE 25 µM (GS 39) vs Nil	4.113	1.185	3.662	0.990	No
INCYDE 50 µM (GS 61) vs DMSO Control (GS 39, 51, 61, 65)	3.383	0.975	3.662	0.998	No
INCYDE 50 µM (GS 61) vs DMSO Control (GS 61, 65, 65+13d)	3.033	0.874	3.662	0.999	No
INCYDE 50 µM (GS 61) vs Nil	2.133	0.615	3.662	1.000	No
TDZ-K 10 µM (GS 61, 65, 65+13d) vs DMSO Control (GS 39, 51, 61, 65)	2.483	0.715	3.662	1.000	No
TDZ-K 10 µM (GS 61, 65, 65+13d) vs DMSO Control (GS 61, 65, 65+13d)	2.133	0.615	3.662	1.000	No
TDZ-K 10 µM (GS 61, 65, 65+13d) vs Nil	1.233	0.355	3.662	1.000	No
INCYDE 50 µM (GS 65) vs DMSO Control (GS 39, 51, 61, 65)	2.077	0.598	3.662	1.000	No
INCYDE 50 µM (GS 65) vs DMSO Control (GS 61, 65, 65+13d)	1.727	0.497	3.662	1.000	No
INCYDE 50 µM (GS 65) vs Nil	0.827	0.238	3.662	1.000	No
INCYDE 25 µM (GS 39, 51, 61, 65) vs DMSO Control (GS 39, 51, 61, 65)	1.407	0.405	3.662	1.000	No
INCYDE 25 µM (GS 39, 51, 61, 65) vs DMSO Control (GS 61, 65, 65+13d)	1.057	0.304	3.662	1.000	No
INCYDE 25 µM (GS 39, 51, 61, 65) vs Nil	0.157	0.045	3.662	1.000	No
Nil vs INCYDE 25 µM (GS 61)	8.177	2.356	3.662	0.503	No
DMSO Control (GS 61, 65, 65+13d) vs INCYDE 25 µM (GS 61)	7.277	2.096	3.662	0.665	No
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 61)	6.927	1.996	3.662	0.726	No
Nil vs TDZ-K 25 µM (GS 61, 65, 65+13d)	7.860	2.264	3.662	0.559	No
DMSO Control (GS 61, 65, 65+13d) vs TDZ-K 25 µM (GS 61, 65, 65+13d)	6.960	2.005	3.662	0.720	No
DMSO Control (GS 39, 51, 61, 65) vs TDZ-K 25 µM (GS 61, 65, 65+13d)	6.610	1.904	3.662	0.778	No
Nil vs INCYDE 10 µM (GS 65)	3.817	1.100	3.662	0.995	No
DMSO Control (GS 61, 65, 65+13d) vs INCYDE 10 µM (GS 65)	2.917	0.840	3.662	1.000	No
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 10 µM (GS 65)	2.567	0.739	3.662	1.000	No
Nil vs INCYDE 25 µM (GS 65)	3.153	0.908	3.662	0.999	No
DMSO Control (GS 61, 65, 65+13d) vs INCYDE 25 µM (GS 65)	2.253	0.649	3.662	1.000	No
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 65)	1.903	0.548	3.662	1.000	No
Nil vs INCYDE 25 µM (GS 51)	2.900	0.835	3.662	1.000	No
DMSO Control (GS 61, 65, 65+13d) vs INCYDE 25 µM (GS 51)	2.000	0.576	3.662	1.000	No
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 51)	1.650	0.475	3.662	1.000	No
Tukey's d critical value:			5.179		
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
INCYDE 25 µM (GS 39)	49.550	2.454	44.484	54.616	A

INCYDE 50 µM (GS 61)	47.570	2.454	42.504	52.636	A
TDZ-K 10 µM (GS 61, 65, 65+13d)	46.670	2.454	41.604	51.736	A
INCYDE 50 µM (GS 65)	46.263	2.454	41.198	51.329	A
INCYDE 25 µM (GS 39, 51, 61, 65)	45.593	2.454	40.528	50.659	A
Nil	45.437	2.454	40.371	50.502	A
DMSO Control (GS 61, 65, 65+13d)	44.537	2.454	39.471	49.602	A
DMSO Control (GS 39, 51, 61, 65)	44.187	2.454	39.121	49.252	A
INCYDE 25 µM (GS 51)	42.537	2.454	37.471	47.602	A
INCYDE 25 µM (GS 65)	42.283	2.454	37.218	47.349	A
INCYDE 10 µM (GS 65)	41.620	2.454	36.554	46.686	A
TDZ-K 25 µM (GS 61, 65, 65+13d)	37.577	2.454	32.511	42.642	A
INCYDE 25 µM (GS 61)	37.260	2.454	32.194	42.326	A

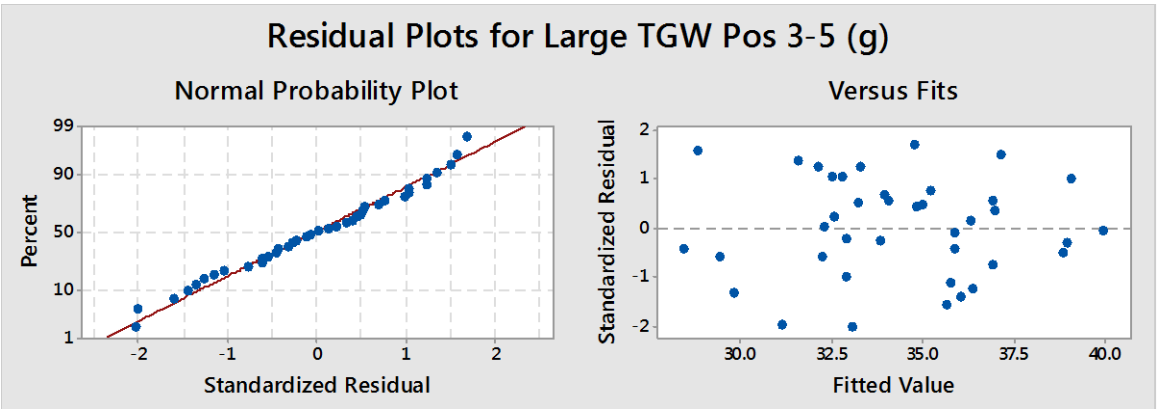
3.4.17 Orator wheat 2013/14: Small TGW Pos 1-2



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	87.83	43.92	1.11	0.347
Treatment	12	201.15	16.76	0.42	0.939
Error	24	951.22	39.63		
Total	38	1240.21			

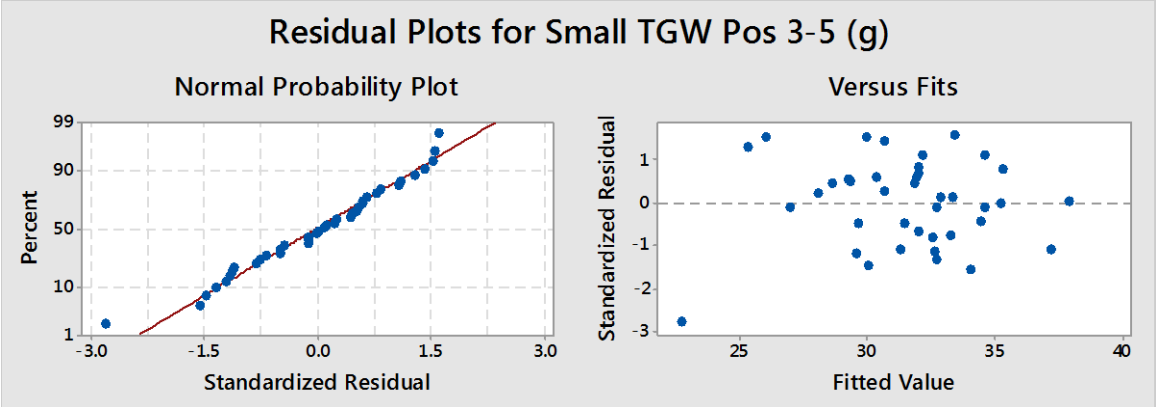
3.4.18 Orator wheat 2013/14: Large TGW Pos 3-5



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	117.9	58.93	3.34	0.053
Treatment	12	186.9	15.57	0.88	0.575
Error	24	423.8	17.66		
Total	38	728.6			

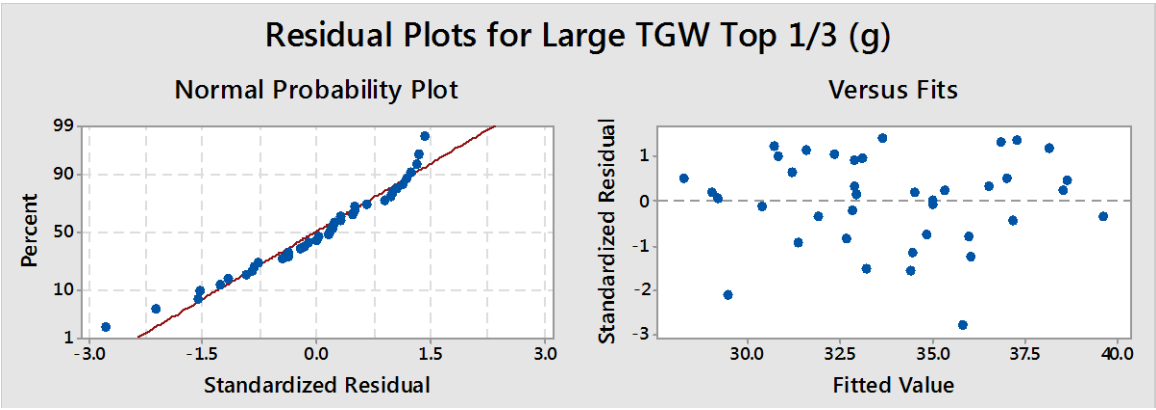
3.4.19 Orator wheat 2013/14: Small TGW Pos 3-5



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	81.32	40.66	1.71	0.203
Treatment	12	284.34	23.70	0.99	0.483
Error	24	572.23	23.84		
Total	38	937.89			

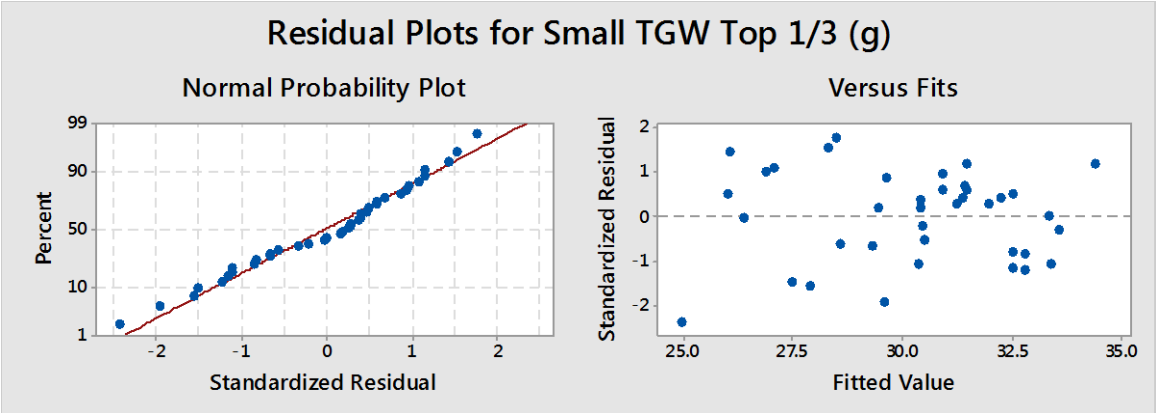
3.4.20 Orator wheat 2013/14: Large TGW Top 1/3



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	87.91	43.95	2.03	0.153
Treatment	12	235.44	19.62	0.91	0.554
Error	24	519.20	21.63		
Total	38	842.54			

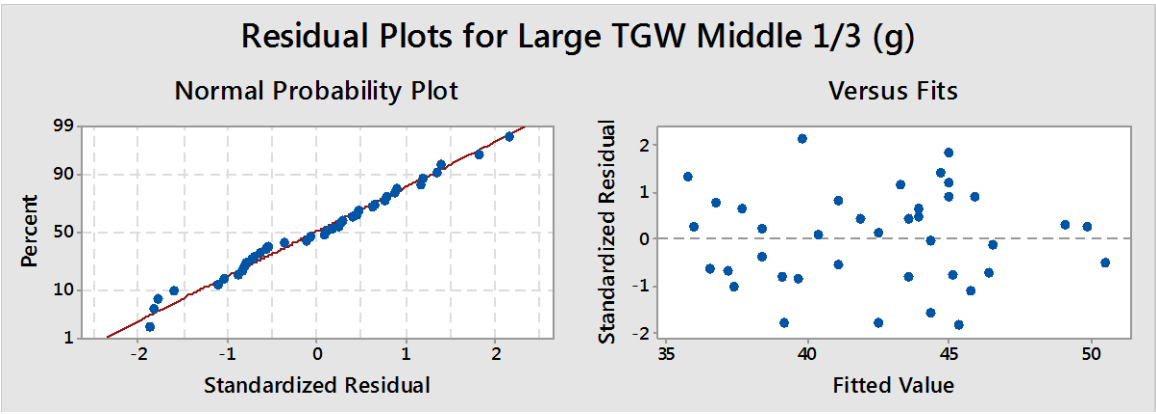
3.4.21 Orator wheat 2013/14: Small TGW Top 1/3



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	23.52	11.76	0.50	0.614
Treatment	12	195.62	16.30	0.69	0.744
Error	24	566.42	23.60		
Total	38	785.56			

3.4.22 Orator wheat 2013/14: Large TGW Middle 1/3



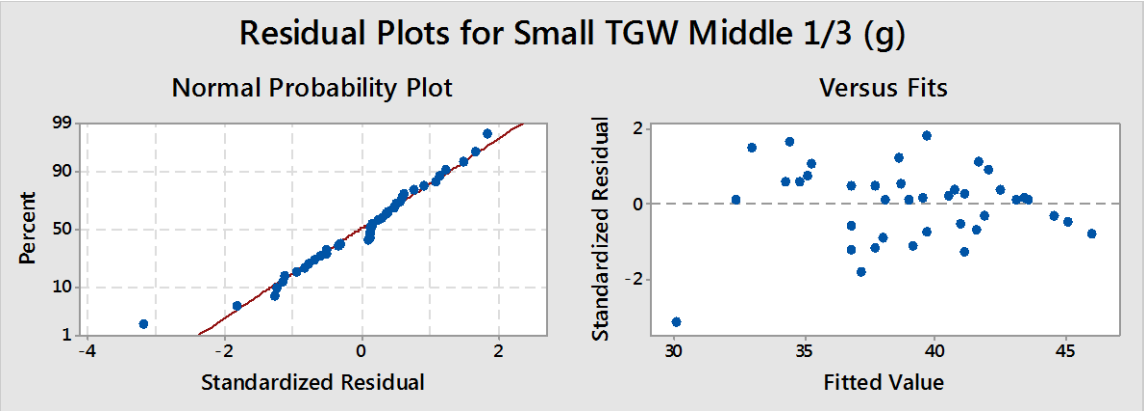
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	12.80	6.398	0.33	0.722
Treatment	12	567.33	47.278	2.44	**0.030**
Error	24	465.18	19.383		
Total	38	1045.31			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
INCYDE 25 µM (GS 39) vs Nil	11.423	3.178	3.662	0.134	No	
INCYDE 25 µM (GS 39) vs DMSO Control (GS 61, 65, 65+13d)	6.560	1.825	3.662	0.819	No	
INCYDE 25 µM (GS 39) vs DMSO Control (GS 39, 51, 61, 65)	5.493	1.528	3.662	0.935	No	
TDZ-K 10 µM (GS 61, 65, 65+13d) vs Nil	7.463	2.076	3.662	0.677	No	
TDZ-K 10 µM (GS 61, 65, 65+13d) vs DMSO Control (GS 61, 65, 65+13d)	2.600	0.723	3.662	1.000	No	
TDZ-K 10 µM (GS 61, 65, 65+13d) vs DMSO Control (GS 39, 51, 61, 65)	1.533	0.427	3.662	1.000	No	
INCYDE 50 µM (GS 61) vs Nil	7.350	2.045	3.662	0.697	No	
INCYDE 50 µM (GS 61) vs DMSO Control (GS 61, 65, 65+13d)	2.487	0.692	3.662	1.000	No	
INCYDE 50 µM (GS 61) vs DMSO Control (GS 39, 51, 61, 65)	1.420	0.395	3.662	1.000	No	
INCYDE 50 µM (GS 65) vs Nil	6.260	1.741	3.662	0.859	No	
INCYDE 50 µM (GS 65) vs DMSO Control (GS 61, 65, 65+13d)	1.397	0.389	3.662	1.000	No	
INCYDE 50 µM (GS 65) vs DMSO Control (GS 39, 51, 61, 65)	0.330	0.092	3.662	1.000	No	
DMSO Control (GS 39, 51, 61, 65) vs TDZ-K 25 µM (GS 61, 65, 65+13d)	7.813	2.174	3.662	0.617	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 10 µM (GS 65)	7.583	2.110	3.662	0.657	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 61)	5.180	1.441	3.662	0.956	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 51)	3.943	1.097	3.662	0.995	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 65)	2.487	0.692	3.662	1.000	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 39, 51, 61, 65)	0.003	0.001	3.662	1.000	No	
INCYDE 25 µM (GS 39, 51, 61, 65) vs Nil	5.927	1.649	3.662	0.896	No	
INCYDE 25 µM (GS 39, 51, 61, 65) vs DMSO Control (GS 61, 65, 65+13d)	1.063	0.296	3.662	1.000	No	
DMSO Control (GS 61, 65, 65+13d) vs TDZ-K 25 µM (GS 61, 65, 65+13d)	6.747	1.877	3.662	0.793	No	
DMSO Control (GS 61, 65, 65+13d) vs INCYDE 10 µM (GS 65)	6.517	1.813	3.662	0.825	No	
DMSO Control (GS 61, 65, 65+13d) vs INCYDE 25 µM (GS 61)	4.113	1.144	3.662	0.992	No	
DMSO Control (GS 61, 65, 65+13d) vs INCYDE 25 µM (GS 51)	2.877	0.800	3.662	1.000	No	
DMSO Control (GS 61, 65, 65+13d) vs INCYDE 25 µM (GS 65)	1.420	0.395	3.662	1.000	No	
INCYDE 25 µM (GS 65) vs Nil	3.443	0.958	3.662	0.998	No	
INCYDE 25 µM (GS 51) vs Nil	1.987	0.553	3.662	1.000	No	
INCYDE 25 µM (GS 61) vs Nil	0.750	0.209	3.662	1.000	No	
Nil vs TDZ-K 25 µM (GS 61, 65, 65+13d)	1.883	0.524	3.662	1.000	No	
Nil vs INCYDE 10 µM (GS 65)	1.653	0.460	3.662	1.000	No	
Tukey's d critical value:			5.179			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
INCYDE 25 µM (GS 39)	49.813	2.542	44.567	55.059	A	
TDZ-K 10 µM (GS 61, 65, 65+13d)	45.853	2.542	40.607	51.099	A	B

INCYDE 50 µM (GS 61)	45.740	2.542	40.494	50.986	A	B
INCYDE 50 µM (GS 65)	44.650	2.542	39.404	49.896	A	B
DMSO Control (GS 39, 51, 61, 65)	44.320	2.542	39.074	49.566	A	B
INCYDE 25 µM (GS 39, 51, 61, 65)	44.317	2.542	39.071	49.563	A	B
DMSO Control (GS 61, 65, 65+13d)	43.253	2.542	38.007	48.499	A	B
INCYDE 25 µM (GS 65)	41.833	2.542	36.587	47.079	A	B
INCYDE 25 µM (GS 51)	40.377	2.542	35.131	45.623	A	B
INCYDE 25 µM (GS 61)	39.140	2.542	33.894	44.386	A	B
Nil	38.390	2.542	33.144	43.636	A	B
INCYDE 10 µM (GS 65)	36.737	2.542	31.491	41.983	A	B
TDZ-K 25 µM (GS 61, 65, 65+13d)	36.507	2.542	31.261	41.753		B

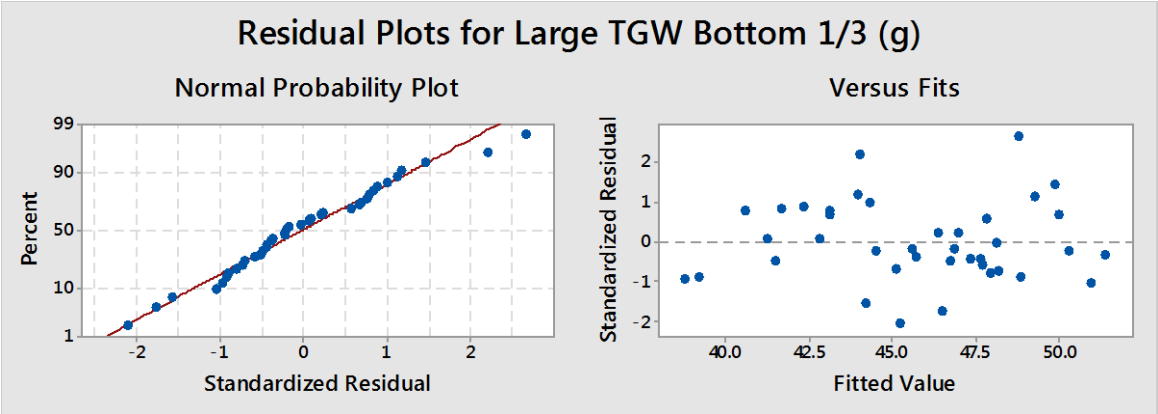
3.4.23 Orator wheat 2013/14: Small TGW Middle 1/3



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	129.4	64.71	2.55	0.099
Treatment	12	382.9	31.91	1.26	0.303
Error	24	608.0	25.33		
Total	38	1120.3			

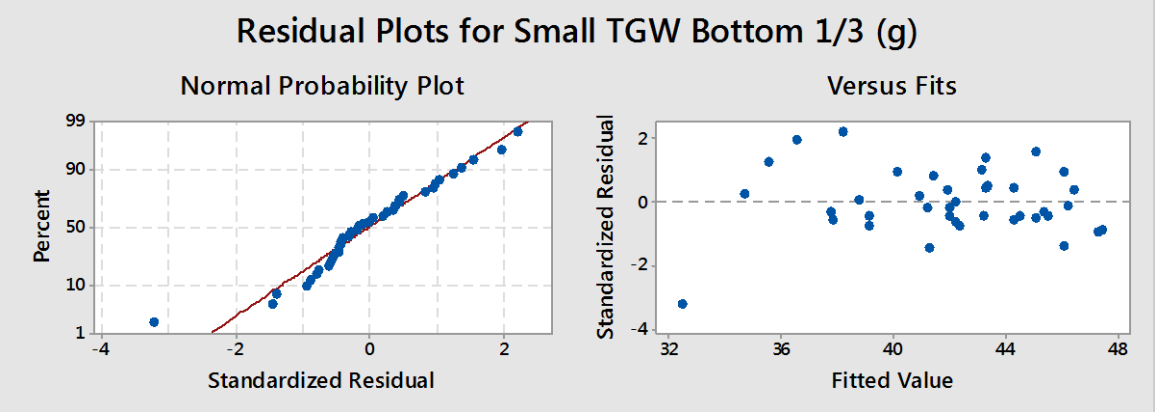
3.4.24 Orator wheat 2013/14: Large TGW Bottom 1/3



Analysis of Variance

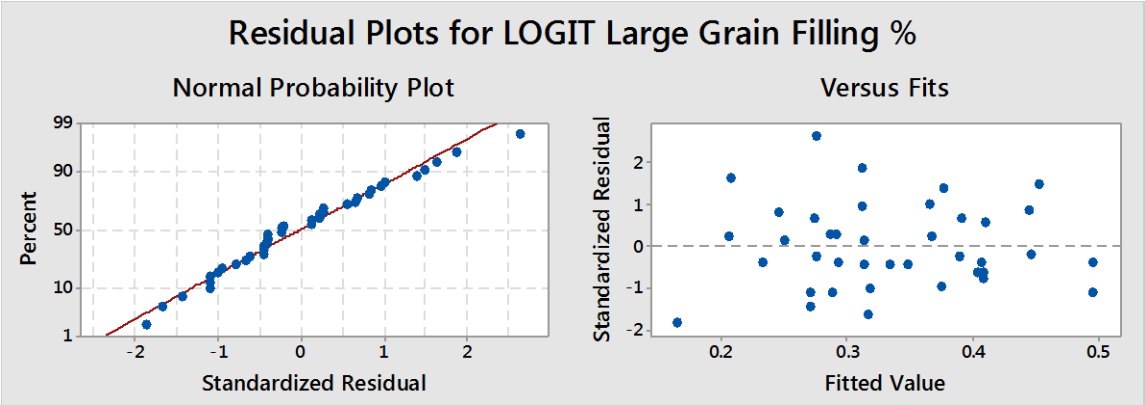
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	88.36	44.18	3.61	0.043
Treatment	12	313.54	26.13	2.14	0.055
Error	24	293.65	12.24		
Total	38	695.55			

3.4.25 Orator wheat 2013/14: Small TGW Bottom 1/3



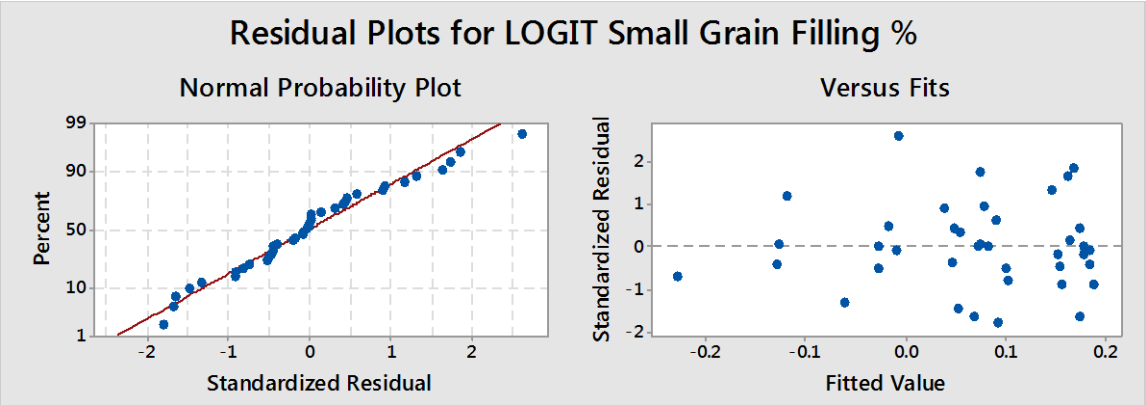
Analysis of Variance					
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	120.1	60.07	2.24	0.128
Treatment	12	378.0	31.50	1.18	0.353
Error	24	642.8	26.78		
Total	38	1140.9			

3.4.26 Orator wheat 2013/14: Large Grain Filling (%)



Analysis of Variance					
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.01534	0.007668	0.40	0.674
Treatment	12	0.23057	0.019214	1.01	0.473
Error	24	0.45817	0.019091		
Total	38	0.70408			

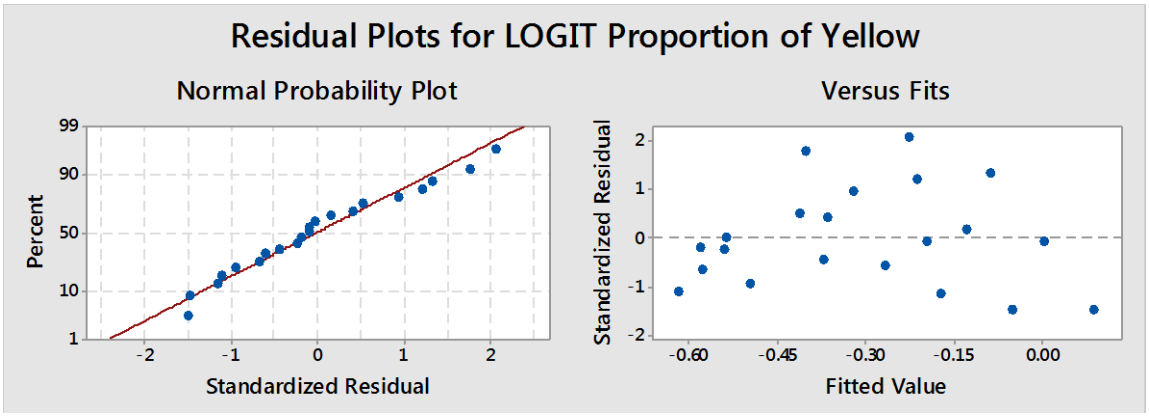
3.4.27 Orator wheat 2013/14: Small Grain Filling (%)



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.09640	0.04820	1.58	0.227
Treatment	12	0.31254	0.02604	0.85	0.601
Error	24	0.73373	0.03057		
Total	38	1.14267			

3.4.28 Orator wheat 2013/14: Proportion of yellow from *Septoria*

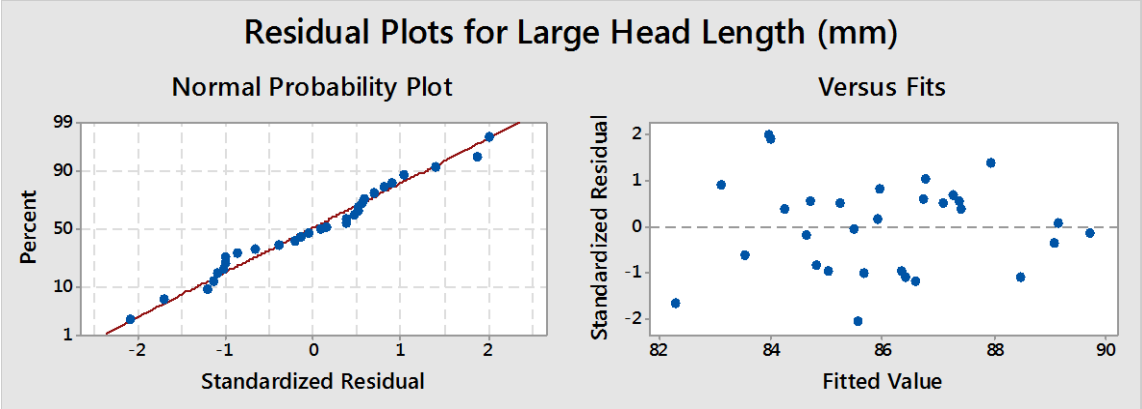


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.1867	0.09337	0.89	0.435
Treatment	6	0.6846	0.11411	1.09	0.421
Error	12	1.2565	0.10471		
Total	20	2.1279			

3.5 Torch wheat 2014/15

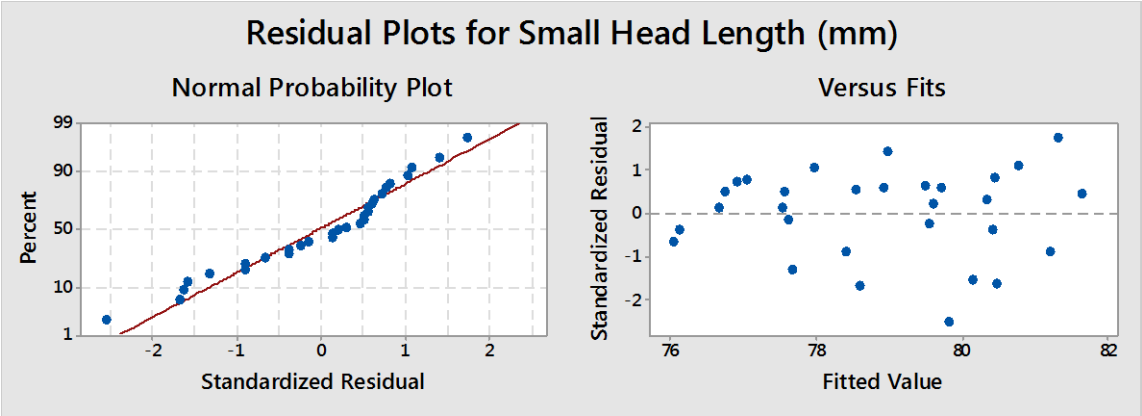
3.5.1 Large Head Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	29.92	14.961	2.92	0.080
Treatment	9	68.46	7.607	1.48	0.228
Error	18	92.36	5.131		
Total	29	190.75			

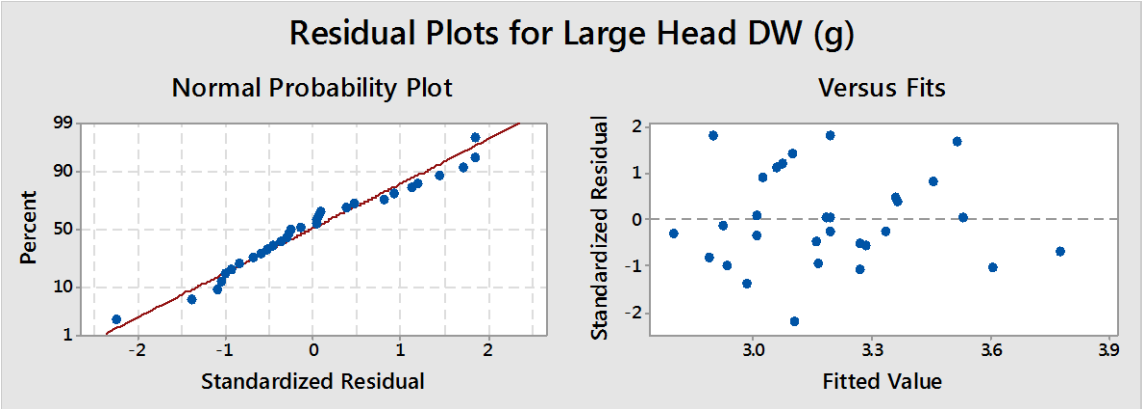
3.5.2 Torch wheat 2014/15: Small Head Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	11.36	5.681	0.59	0.564
Treatment	9	65.44	7.271	0.76	0.655
Error	18	172.73	9.596		
Total	29	249.53			

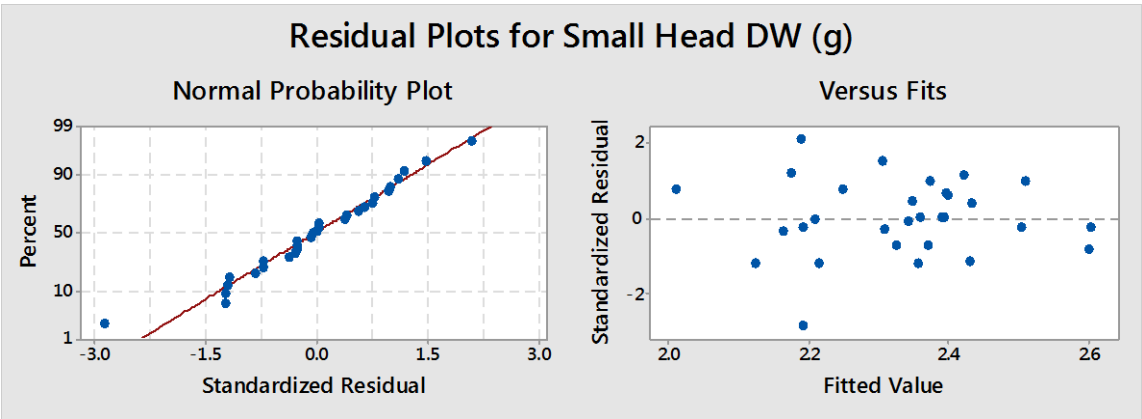
3.5.3 Torch wheat 2014/15: Large Head DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.3524	0.17620	2.38	0.121
Treatment	9	1.2221	0.13579	1.83	0.131
Error	18	1.3342	0.07412		
Total	29	2.9087			

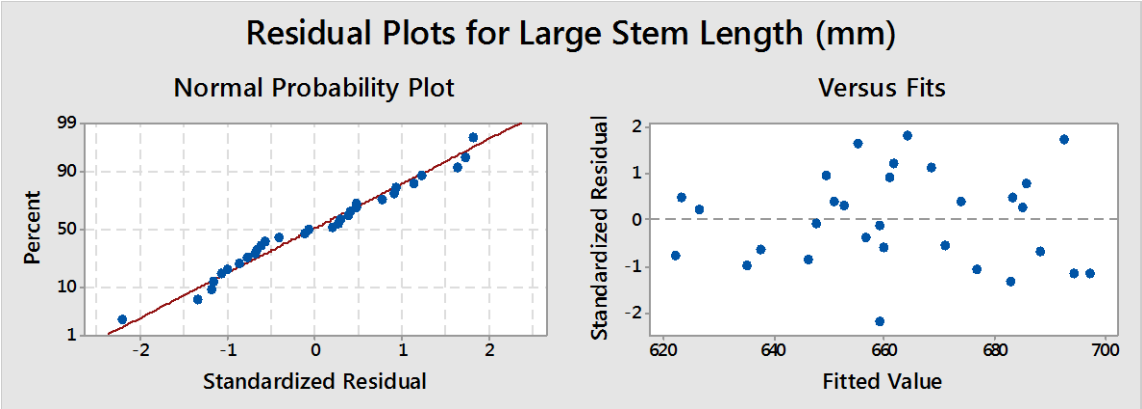
3.5.4 Torch wheat 2014/15: Small Head DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.2216	0.11079	1.92	0.176
Treatment	9	0.3322	0.03691	0.64	0.750
Error	18	1.0395	0.05775		
Total	29	1.5933			

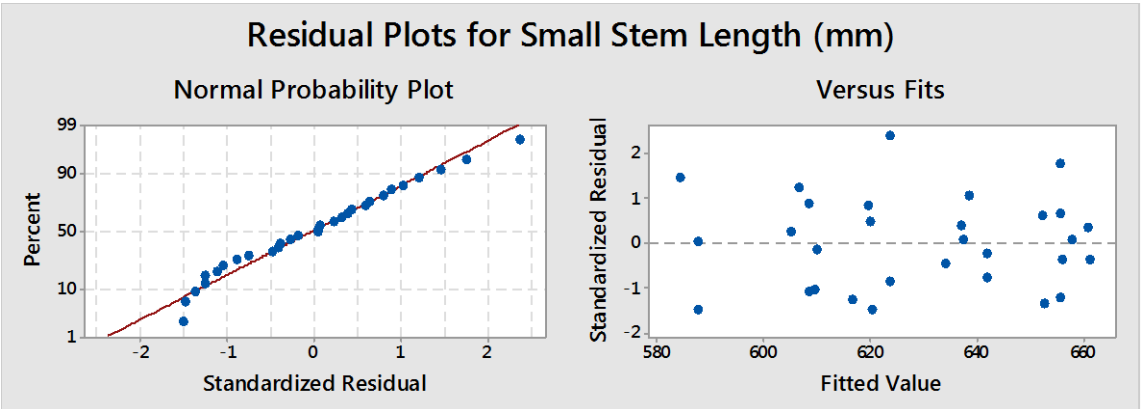
3.5.5 Torch wheat 2014/15: Large Stem Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	5923	2961.4	2.45	0.115
Treatment	9	7009	778.8	0.64	0.746
Error	18	21767	1209.3		
Total	29	34699			

3.5.6 Torch wheat 2014/15: Small Stem Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	68.5	34.26	0.02	0.985
Treatment	9	15887.1	1765.24	0.80	0.620
Error	18	39643.8	2202.43		
Total	29	55599.5			

3.5.7 Torch wheat 2014/15: Tiller no.

Poisson Regression Analysis: Tiller No. versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	11	1.9394	33.13%	1.9394	0.17631	1.94	0.999
Block	2	0.1910	3.26%	0.1910	0.09549	0.19	0.909
Treatment	9	1.7485	29.86%	1.7485	0.19427	1.75	0.995
Error	18	3.9154	66.87%	3.9154	0.21752		
Total	29	5.8549	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value
Constant	1.123	0.357	(0.424, 1.823)	3.15
Block				
Block1	0.000000	0.000000	(0.000000, 0.000000)	*
Block2	0.063	0.240	(-0.407, 0.533)	0.26
Block3	-0.042	0.246	(-0.524, 0.440)	-0.17
Treatment				
CPPU 10 µM (GS 51, 65)	0.186	0.444	(-0.684, 1.055)	0.42
CPPU 10 µM (GS 61, 65)	0.212	0.441	(-0.652, 1.077)	0.48
CPPU 100 µM (GS 51, 65)	0.216	0.441	(-0.648, 1.080)	0.49
CPPU 100 µM (GS 61, 65)	-0.138	0.481	(-1.080, 0.804)	-0.29
CPPU 30 µM (GS 51, 65)	0.042	0.459	(-0.857, 0.942)	0.09
CPPU 30 µM (GS 61, 65)	0.168	0.445	(-0.705, 1.041)	0.38
DMSO Control (GS 51, 61, 65, 65+15d)	0.000000	0.000000	(0.000000, 0.000000)	*
Nil	0.000	0.464	(-0.909, 0.909)	0.00
TDZ-K 10 µM (GS 61, 65, 65+15d)	0.255	0.437	(-0.601, 1.111)	0.58
TDZ-K 50 µM (GS 61, 65, 65+15d)	-0.093	0.475	(-1.023, 0.838)	-0.20

Term	*P*-Value	VIF
Constant	0.002	
Block		
Block1	*	*
Block2	0.792	1.34
Block3	0.865	1.34
Treatment		
CPPU 10 µM (GS 51, 65)	0.675	1.96
CPPU 10 µM (GS 61, 65)	0.630	1.98
CPPU 100 µM (GS 51, 65)	0.624	1.99
CPPU 100 µM (GS 61, 65)	0.774	1.72
CPPU 30 µM (GS 51, 65)	0.927	1.85
CPPU 30 µM (GS 61, 65)	0.706	1.95
DMSO Control (GS 51, 61, 65, 65+15d)	*	*
Nil	1.000	1.82
TDZ-K 10 µM (GS 61, 65, 65+15d)	0.560	2.02
TDZ-K 50 µM (GS 61, 65, 65+15d)	0.845	1.75

Regression Equation

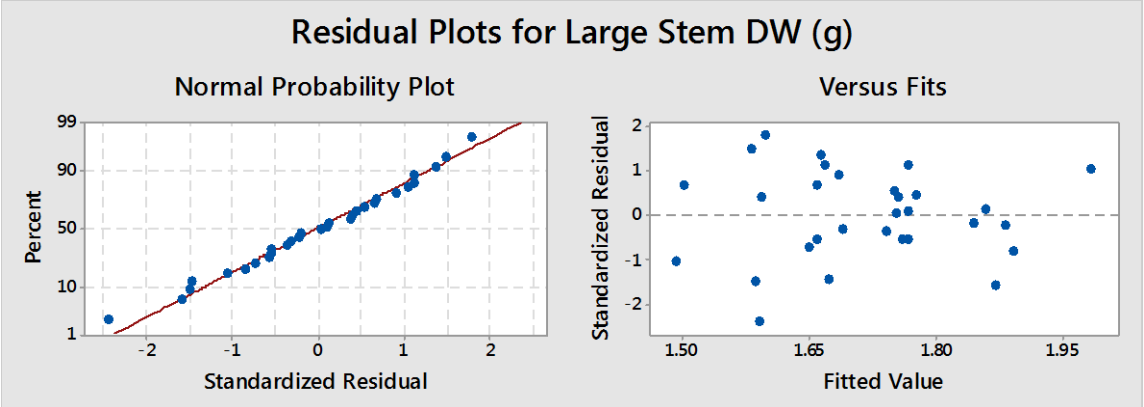
Tiller No. = exp(Y')

Y' = 1.123 +0.0Block_Block1 +0.063Block_Block2 -0.042 Block_Block3
+0.186Treatment_CPPU 10 µM (GS 51, 65) +0.212Treatment_CPPU 10 µM (GS 61, 65)
+0.216Treatment_CPPU 100 µM (GS 51, 65) -0.138Treatment_CPPU 100 µM (GS 61, 65)
+0.042Treatment_CPPU 30 µM (GS 51, 65) +0.168Treatment_CPPU 30 µM (GS 61, 65)
+0.0Treatment_DMSO Control (GS 51, 61, 65, 65+15d) +0.000Treatment_Nil
+0.255Treatment_TDZ-K 10 µM (GS 61, 65, 65+15d) -0.093Treatment_TDZ-K 50 µM (GS 61, 65, 65+15d)

Goodness-o*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	18	3.91543	0.21752	3.92	1.000
Pearson	18	3.80115	0.21118	3.80	1.000

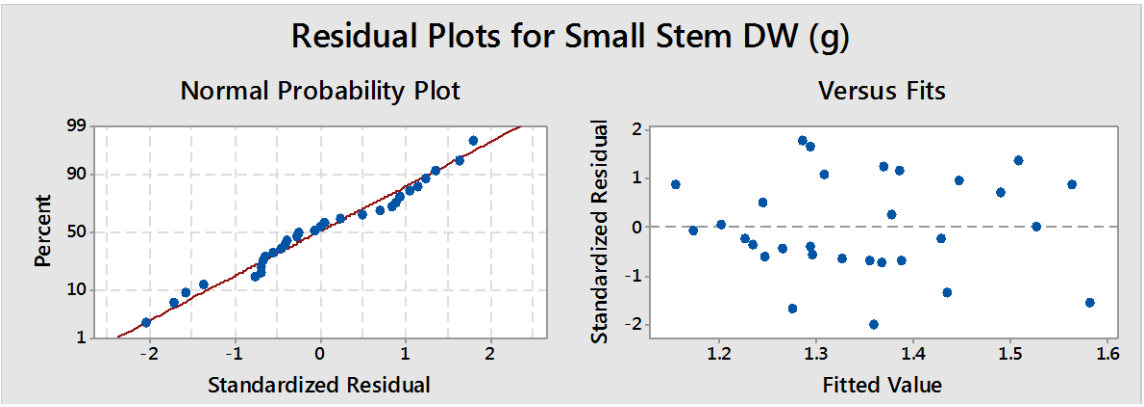
3.5.8 Torch wheat 2014/15: Large Stem DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.06442	0.03221	1.60	0.230
Treatment	9	0.33978	0.03775	1.87	0.123
Error	18	0.36324	0.02018		
Total	29	0.76744			

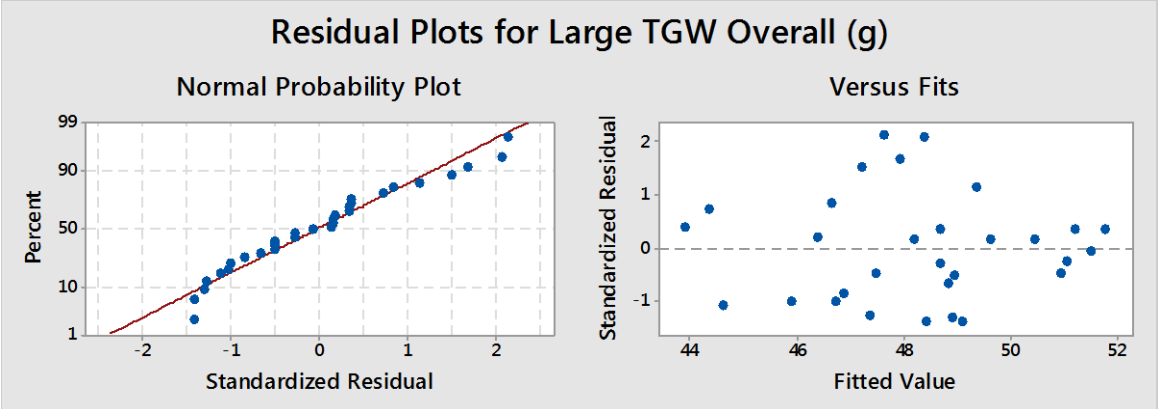
3.5.9 Torch wheat 2014/15: Small Stem DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.04798	0.02399	1.42	0.269
Treatment	9	0.32799	0.03644	2.15	0.080
Error	18	0.30514	0.01695		
Total	29	0.68111			

3.5.10 Torch wheat 2014/15: Large TGW Overall

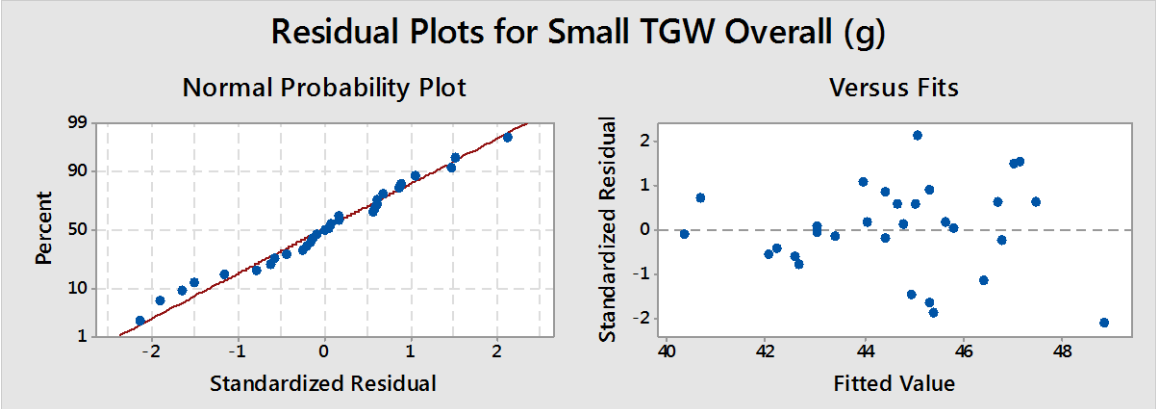


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	2.851	1.425	0.92	0.417
Treatment	9	120.970	13.441	8.67	**0.000**
Error	18	27.919	1.551		
Total	29	151.740			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:								
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant			
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 30 µM (GS 61, 65)	7.166	7.047	3.585	< 0.0001	Yes			
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 30 µM (GS 51, 65)	5.157	5.071	3.585	0.002	Yes			
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 100 µM (GS 61, 65)	4.318	4.246	3.585	0.013	Yes			
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 10 µM (GS 61, 65)	4.172	4.103	3.585	0.018	Yes			
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 100 µM (GS 51, 65)	2.860	2.813	3.585	0.204	No			
DMSO Control (GS 51, 61, 65, 65+15d) vs TDZ-K 50 µM (GS 61, 65, 65+15d)	2.680	2.636	3.585	0.270	No			
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 10 µM (GS 51, 65)	2.148	2.112	3.585	0.542	No			
DMSO Control (GS 51, 61, 65, 65+15d) vs TDZ-K 10 µM (GS 61, 65, 65+15d)	0.586	0.576	3.585	1.000	No			
TDZ-K 10 µM (GS 61, 65, 65+15d) vs Nil	2.517	2.475	3.585	0.342	No			
CPPU 10 µM (GS 51, 65) vs Nil	0.954	0.939	3.585	0.993	No			
TDZ-K 50 µM (GS 61, 65, 65+15d) vs Nil	0.422	0.415	3.585	1.000	No			
CPPU 100 µM (GS 51, 65) vs Nil	0.242	0.238	3.585	1.000	No			
Nil vs CPPU 30 µM (GS 61, 65)	4.063	3.996	3.585	0.022	Yes			
Nil vs CPPU 30 µM (GS 51, 65)	2.054	2.020	3.585	0.597	No			
Nil vs CPPU 100 µM (GS 61, 65)	1.216	1.195	3.585	0.964	No			
Nil vs CPPU 10 µM (GS 61, 65)	1.070	1.052	3.585	0.984	No			
Tukey's d critical value:			5.071					
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
DMSO Control (GS 51, 61, 65, 65+15d)	51.466	0.719	49.955	52.976	A			
TDZ-K 10 µM (GS 61, 65, 65+15d)	50.880	0.719	49.369	52.391	A	B		
CPPU 10 µM (GS 51, 65)	49.318	0.719	47.807	50.828	A	B	C	
TDZ-K 50 µM (GS 61, 65, 65+15d)	48.786	0.719	47.275	50.296	A	B	C	
CPPU 100 µM (GS 51, 65)	48.606	0.719	47.095	50.116	A	B	C	
Nil	48.363	0.719	46.853	49.874	A	B	C	
CPPU 10 µM (GS 61, 65)	47.293	0.719	45.783	48.804		B	C	D
CPPU 100 µM (GS 61, 65)	47.148	0.719	45.637	48.658			C	D
CPPU 30 µM (GS 51, 65)	46.309	0.719	44.798	47.820			C	D
CPPU 30 µM (GS 61, 65)	44.300	0.719	42.789	45.811				D

3.5.11 Torch wheat 2014/15: Small TGW Overall



Analysis of Variance

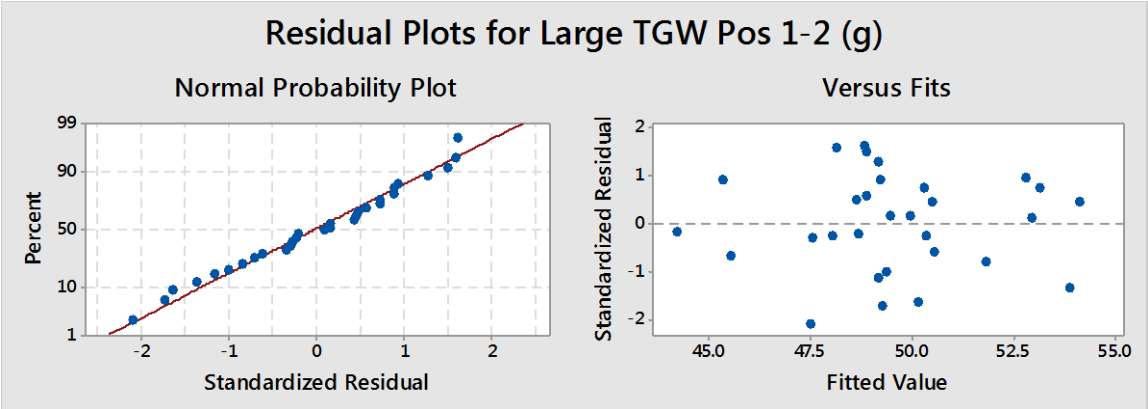
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	17.15	8.577	2.05	0.158
Treatment	9	100.06	11.117	2.65	**0.037**
Error	18	75.47	4.193		
Total	29	192.68			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Small TGW Overall (g)):						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
CPPU 100 µM (GS 51, 65) vs CPPU 10 µM (GS 61, 65)	6.812	4.075	3.585	**0.019**	Yes	
CPPU 100 µM (GS 51, 65) vs CPPU 30 µM (GS 61, 65)	4.918	2.941	3.585	0.164	No	
CPPU 100 µM (GS 51, 65) vs CPPU 30 µM (GS 51, 65)	4.449	2.661	3.585	0.259	No	
CPPU 100 µM (GS 51, 65) vs Nil	4.101	2.453	3.585	0.353	No	
CPPU 100 µM (GS 51, 65) vs TDZ-K 50 µM (GS 61, 65, 65+15d)	3.070	1.836	3.585	0.707	No	
CPPU 100 µM (GS 51, 65) vs TDZ-K 10 µM (GS 61, 65, 65+15d)	2.459	1.471	3.585	0.887	No	
CPPU 100 µM (GS 51, 65) vs CPPU 10 µM (GS 51, 65)	2.173	1.300	3.585	0.941	No	
CPPU 100 µM (GS 51, 65) vs CPPU 100 µM (GS 61, 65)	2.080	1.244	3.585	0.954	No	
CPPU 100 µM (GS 51, 65) vs DMSO Control (GS 51, 61, 65, 65+15d)	1.833	1.097	3.585	0.979	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 10 µM (GS 61, 65)	4.979	2.978	3.585	0.154	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 30 µM (GS 61, 65)	3.084	1.845	3.585	0.702	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 30 µM (GS 51, 65)	2.616	1.564	3.585	0.848	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs Nil	2.268	1.356	3.585	0.926	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs TDZ-K 50 µM (GS 61, 65, 65+15d)	1.237	0.740	3.585	0.999	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs TDZ-K 10 µM (GS 61, 65, 65+15d)	0.626	0.374	3.585	1.000	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 10 µM (GS 51, 65)	0.340	0.203	3.585	1.000	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 100 µM (GS 61, 65)	0.247	0.148	3.585	1.000	No	
CPPU 100 µM (GS 61, 65) vs CPPU 10 µM (GS 61, 65)	4.732	2.830	3.585	0.198	No	
CPPU 100 µM (GS 61, 65) vs CPPU 30 µM (GS 61, 65)	2.838	1.697	3.585	0.784	No	
CPPU 100 µM (GS 61, 65) vs CPPU 30 µM (GS 51, 65)	2.369	1.417	3.585	0.906	No	

CPPU 100 µM (GS 61, 65) vs Nil	2.021	1.209	3.585	0.961	No	
CPPU 100 µM (GS 61, 65) vs TDZ-K 50 µM (GS 61, 65, 65+15d)	0.990	0.592	3.585	1.000	No	
CPPU 100 µM (GS 61, 65) vs TDZ-K 10 µM (GS 61, 65, 65+15d)	0.379	0.227	3.585	1.000	No	
CPPU 100 µM (GS 61, 65) vs CPPU 10 µM (GS 51, 65)	0.093	0.056	3.585	1.000	No	
CPPU 10 µM (GS 51, 65) vs CPPU 10 µM (GS 61, 65)	4.639	2.775	3.585	0.217	No	
CPPU 10 µM (GS 51, 65) vs CPPU 30 µM (GS 61, 65)	2.744	1.642	3.585	0.812	No	
CPPU 10 µM (GS 51, 65) vs CPPU 30 µM (GS 51, 65)	2.276	1.361	3.585	0.924	No	
CPPU 10 µM (GS 51, 65) vs Nil	1.928	1.153	3.585	0.971	No	
CPPU 10 µM (GS 51, 65) vs TDZ-K 50 µM (GS 61, 65, 65+15d)	0.897	0.536	3.585	1.000	No	
CPPU 10 µM (GS 51, 65) vs TDZ-K 10 µM (GS 61, 65, 65+15d)	0.286	0.171	3.585	1.000	No	
TDZ-K 10 µM (GS 61, 65, 65+15d) vs CPPU 10 µM (GS 61, 65)	4.353	2.604	3.585	0.283	No	
TDZ-K 10 µM (GS 61, 65, 65+15d) vs CPPU 30 µM (GS 61, 65)	2.459	1.471	3.585	0.887	No	
TDZ-K 10 µM (GS 61, 65, 65+15d) vs CPPU 30 µM (GS 51, 65)	1.990	1.190	3.585	0.965	No	
TDZ-K 10 µM (GS 61, 65, 65+15d) vs Nil	1.642	0.982	3.585	0.990	No	
TDZ-K 10 µM (GS 61, 65, 65+15d) vs TDZ-K 50 µM (GS 61, 65, 65+15d)	0.611	0.366	3.585	1.000	No	
TDZ-K 50 µM (GS 61, 65, 65+15d) vs CPPU 10 µM (GS 61, 65)	3.742	2.238	3.585	0.468	No	
TDZ-K 50 µM (GS 61, 65, 65+15d) vs CPPU 30 µM (GS 61, 65)	1.848	1.105	3.585	0.978	No	
TDZ-K 50 µM (GS 61, 65, 65+15d) vs CPPU 30 µM (GS 51, 65)	1.379	0.825	3.585	0.997	No	
TDZ-K 50 µM (GS 61, 65, 65+15d) vs Nil	1.031	0.617	3.585	1.000	No	
Nil vs CPPU 10 µM (GS 61, 65)	2.711	1.622	3.585	0.822	No	
Nil vs CPPU 30 µM (GS 61, 65)	0.817	0.488	3.585	1.000	No	
Nil vs CPPU 30 µM (GS 51, 65)	0.348	0.208	3.585	1.000	No	
CPPU 30 µM (GS 51, 65) vs CPPU 10 µM (GS 61, 65)	2.363	1.414	3.585	0.908	No	
CPPU 30 µM (GS 51, 65) vs CPPU 30 µM (GS 61, 65)	0.469	0.280	3.585	1.000	No	

CPPU 30 µM (GS 61, 65) vs CPPU 10 µM (GS 61, 65)	1.894	1.133	3.585	0.974	No	
Tukey's d critical value:			5.071			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
CPPU 100 µM (GS 51, 65)	47.850	1.182	45.366	50.334	A	
DMSO Control (GS 51, 61, 65, 65+15d)	46.017	1.182	43.533	48.500	A	B
CPPU 100 µM (GS 61, 65)	45.770	1.182	43.286	48.254	A	B
CPPU 10 µM (GS 51, 65)	45.677	1.182	43.193	48.160	A	B
TDZ-K 10 µM (GS 61, 65, 65+15d)	45.391	1.182	42.907	47.875	A	B
TDZ-K 50 µM (GS 61, 65, 65+15d)	44.780	1.182	42.296	47.264	A	B
Nil	43.749	1.182	41.265	46.233	A	B
CPPU 30 µM (GS 51, 65)	43.401	1.182	40.917	45.885	A	B
CPPU 30 µM (GS 61, 65)	42.932	1.182	40.448	45.416	A	B
CPPU 10 µM (GS 61, 65)	41.038	1.182	38.554	43.522		B
Summary of all pairwise comparisons for Treatment (Tukey (HSD)):						
Category	LS means(Small TGW Overall (g))	Groups				
CPPU 100 µM (GS 51, 65)	47.850	A				
DMSO Control (GS 51, 61, 65, 65+15d)	46.017	A	B			
CPPU 100 µM (GS 61, 65)	45.770	A	B			
CPPU 10 µM (GS 51, 65)	45.677	A	B			
TDZ-K 10 µM (GS 61, 65, 65+15d)	45.391	A	B			
TDZ-K 50 µM (GS 61, 65, 65+15d)	44.780	A	B			
Nil	43.749	A	B			
CPPU 30 µM (GS 51, 65)	43.401	A	B			
CPPU 30 µM (GS 61, 65)	42.932	A	B			
CPPU 10 µM (GS 61, 65)	41.038		B			

3.5.12 Torch wheat 2014/15: Large TGW Pos 1-2

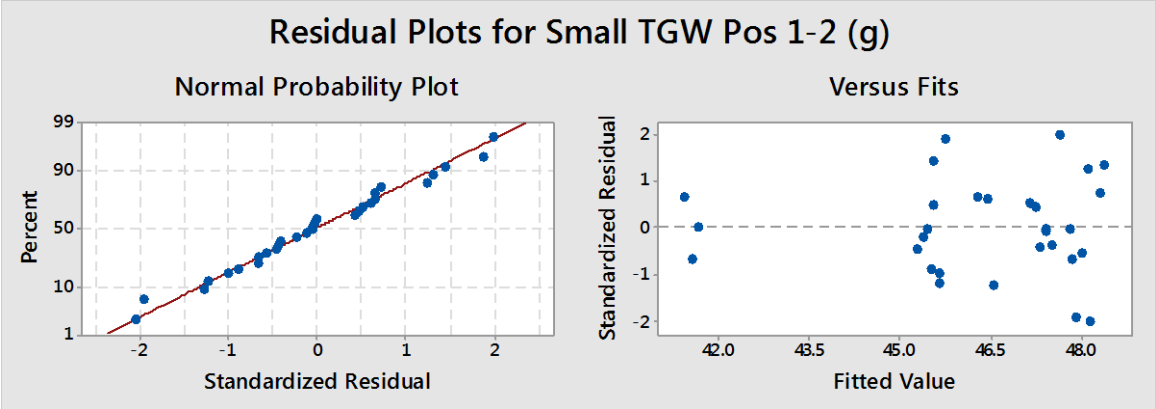


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	10.06	5.028	2.63	0.099
Treatment	9	152.42	16.935	8.87	**0.000**
Error	18	34.37	1.909		
Total	29	196.84			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:								
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant			
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 30 µM (GS 61, 65)	8.588	7.612	3.585	< 0.0001	Yes			
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 10 µM (GS 61, 65)	5.289	4.688	3.585	0.005	Yes			
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 30 µM (GS 51, 65)	5.244	4.648	3.585	0.006	Yes			
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 100 µM (GS 61, 65)	4.741	4.202	3.585	0.015	Yes			
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 100 µM (GS 51, 65)	3.940	3.492	3.585	0.060	No			
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 10 µM (GS 51, 65)	3.607	3.197	3.585	0.104	No			
DMSO Control (GS 51, 61, 65, 65+15d) vs TDZ-K 50 µM (GS 61, 65, 65+15d)	3.558	3.153	3.585	0.113	No			
DMSO Control (GS 51, 61, 65, 65+15d) vs TDZ-K 10 µM (GS 61, 65, 65+15d)	0.956	0.847	3.585	0.996	No			
TDZ-K 10 µM (GS 61, 65, 65+15d) vs Nil	3.683	3.265	3.585	0.092	No			
TDZ-K 50 µM (GS 61, 65, 65+15d) vs Nil	1.081	0.958	3.585	0.991	No			
CPPU 10 µM (GS 51, 65) vs Nil	1.032	0.915	3.585	0.994	No			
CPPU 100 µM (GS 51, 65) vs Nil	0.699	0.619	3.585	1.000	No			
Nil vs CPPU 30 µM (GS 61, 65)	3.949	3.500	3.585	0.059	No			
Nil vs CPPU 10 µM (GS 61, 65)	0.650	0.576	3.585	1.000	No			
Nil vs CPPU 30 µM (GS 51, 65)	0.606	0.537	3.585	1.000	No			
Nil vs CPPU 100 µM (GS 61, 65)	0.102	0.091	3.585	1.000	No			
Tukey's d critical value:			5.071					
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
DMSO Control (GS 51, 61, 65, 65+15d)	53.633	0.798	51.957	55.309	A			
TDZ-K 10 µM (GS 61, 65, 65+15d)	52.678	0.798	51.002	54.354	A	B		
TDZ-K 50 µM (GS 61, 65, 65+15d)	50.076	0.798	48.400	51.752	A	B	C	
CPPU 10 µM (GS 51, 65)	50.027	0.798	48.351	51.703	A	B	C	
CPPU 100 µM (GS 51, 65)	49.693	0.798	48.017	51.369	A	B	C	
Nil	48.994	0.798	47.318	50.670		B	C	D
CPPU 100 µM (GS 61, 65)	48.892	0.798	47.216	50.568		B	C	D
CPPU 30 µM (GS 51, 65)	48.389	0.798	46.713	50.065			C	D
CPPU 10 µM (GS 61, 65)	48.344	0.798	46.668	50.020			C	D
CPPU 30 µM (GS 61, 65)	45.046	0.798	43.370	46.722				D

3.5.13 Torch wheat 2014/15: Small TGW Pos 1-2



Analysis of Variance

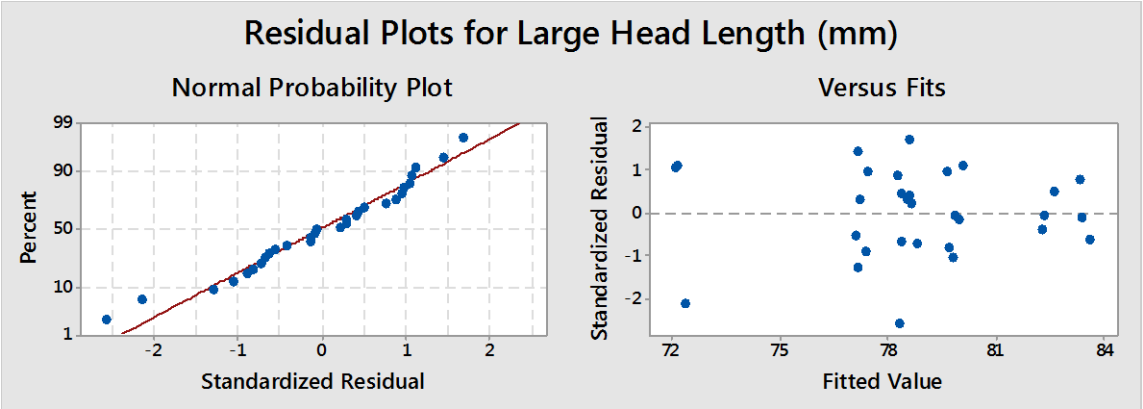
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.334	0.1671	0.05	0.949
Treatment	9	106.894	11.8771	3.76	**0.008**
Error	18	56.871	3.1595		
Total	29	164.099			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Small TGW Pos 1-2 (g)):						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Signifi cant	
CPPU 10 µM (GS 51, 65) vs Nil	2.744	1.891	3.585	0.675	No	
CPPU 10 µM (GS 51, 65) vs DMSO Control (GS 51, 61, 65, 65+15d)	0.291	0.201	3.585	1.000	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 10 µM (GS 61, 65)	6.442	4.439	3.585	**0.009**	Yes	
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 30 µM (GS 51, 65)	2.566	1.768	3.585	0.746	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 30 µM (GS 61, 65)	2.346	1.616	3.585	0.824	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs TDZ-K 10 µM (GS 61, 65, 65+15d)	1.557	1.073	3.585	0.982	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 100 µM (GS 61, 65)	0.687	0.473	3.585	1.000	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs TDZ-K 50 µM (GS 61, 65, 65+15d)	0.590	0.407	3.585	1.000	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs CPPU 100 µM (GS 51, 65)	0.184	0.127	3.585	1.000	No	
CPPU 100 µM (GS 51, 65) vs Nil	2.269	1.563	3.585	0.849	No	
TDZ-K 50 µM (GS 61, 65, 65+15d) vs Nil	1.863	1.284	3.585	0.945	No	
CPPU 100 µM (GS 61, 65) vs Nil	1.767	1.217	3.585	0.960	No	
TDZ-K 10 µM (GS 61, 65, 65+15d) vs Nil	0.897	0.618	3.585	1.000	No	
CPPU 30 µM (GS 61, 65) vs Nil	0.108	0.074	3.585	1.000	No	
Nil vs CPPU 10 µM (GS 61, 65)	3.989	2.748	3.585	0.226	No	
Nil vs CPPU 30 µM (GS 51, 65)	0.112	0.077	3.585	1.000	No	
Tukey's d critical value:			5.071			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
CPPU 10 µM (GS 51, 65)	48.286	1.026	46.130	50.442	A	
DMSO Control (GS 51, 61, 65, 65+15d)	47.994	1.026	45.838	50.150	A	
CPPU 100 µM (GS 51, 65)	47.810	1.026	45.654	49.966	A	
TDZ-K 50 µM (GS 61, 65, 65+15d)	47.404	1.026	45.248	49.560	A	
CPPU 100 µM (GS 61, 65)	47.308	1.026	45.152	49.464	A	
TDZ-K 10 µM (GS 61, 65, 65+15d)	46.438	1.026	44.282	48.594	A	B
CPPU 30 µM (GS 61, 65)	45.649	1.026	43.493	47.805	A	B
Nil	45.541	1.026	43.385	47.697	A	B

CPPU 30 µM (GS 51, 65)	45.429	1.026	43.273	47.585	A	B
CPPU 10 µM (GS 61, 65)	41.552	1.026	39.396	43.708		B
Summary of all pairwise comparisons for Blocks (Tukey (HSD)):						
Category	LS means(Small TGW Pos 1-2 (g))	Groups				
Block1	46.459	A				
Block2	46.362	A				
Block3	46.203	A				
Summary of all pairwise comparisons for Treatment (Tukey (HSD)):						
Category	LS means(Small TGW Pos 1-2 (g))	Groups				
CPPU 10 µM (GS 51, 65)	48.286	A				
DMSO Control (GS 51, 61, 65, 65+15d)	47.994	A				
CPPU 100 µM (GS 51, 65)	47.810	A				
TDZ-K 50 µM (GS 61, 65, 65+15d)	47.404	A				
CPPU 100 µM (GS 61, 65)	47.308	A				
TDZ-K 10 µM (GS 61, 65, 65+15d)	46.438	A	B			
CPPU 30 µM (GS 61, 65)	45.649	A	B			
Nil	45.541	A	B			
CPPU 30 µM (GS 51, 65)	45.429	A	B			
CPPU 10 µM (GS 61, 65)	41.552		B			

3.6 Quench barley 2014/15

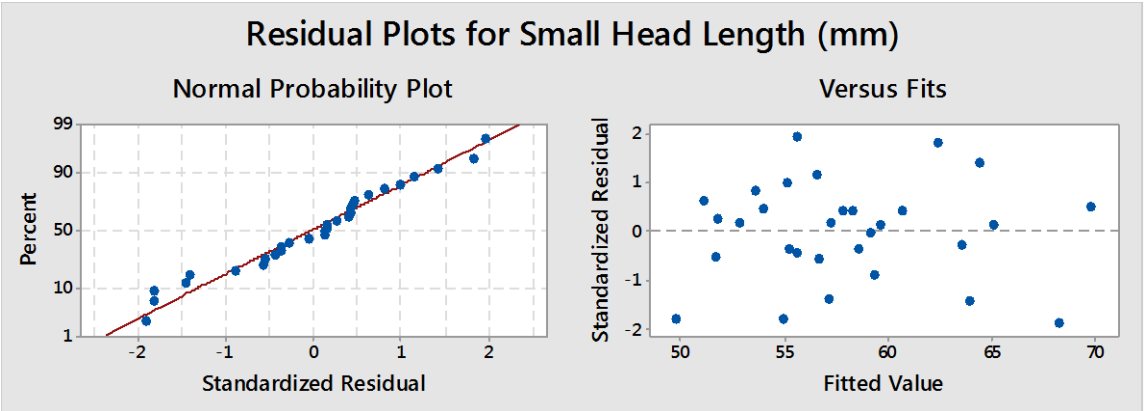
3.6.1 Quench barley 2014/15: Large Head Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.510	0.2548	0.02	0.982
Treatment	9	257.247	28.5829	2.00	0.101
Error	18	257.524	14.3069		
Total	29	515.280			

3.6.2 Quench barley 2014/15: Small Head Length

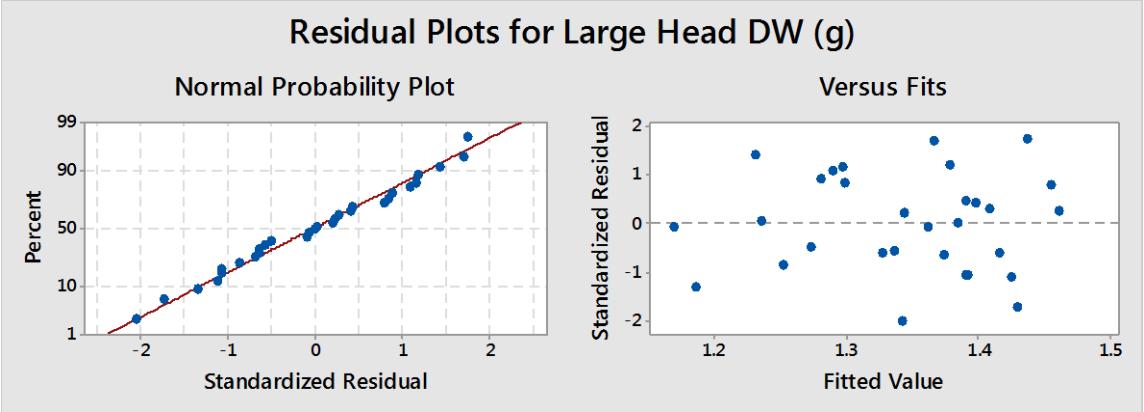


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	157.4	78.70	4.75	0.022
Treatment	9	579.6	64.40	3.88	**0.007**
Error	18	298.4	16.58		
Total	29	1035.4			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
INC 25 µM (GS 65) vs Nil	14.700	4.421	3.585	0.009	Yes	
INC 25 µM (GS 65) vs DMSO Control (GS 51, 61, 65, 65+15d)	4.767	1.434	3.585	0.901	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 10 µM (GS 65)	8.558	2.574	3.585	0.296	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 25 µM (GS 39)	7.933	2.386	3.585	0.387	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 50 µM (GS 65)	7.892	2.374	3.585	0.393	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 50 µM (GS 61)	6.833	2.055	3.585	0.576	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 25 µM (GS 61)	5.700	1.714	3.585	0.775	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 25 µM (GS 51)	4.400	1.323	3.585	0.935	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 25 µM (GS 39, 51, 61, 65)	1.100	0.331	3.585	1.000	No	
INC 25 µM (GS 39, 51, 61, 65) vs Nil	8.833	2.657	3.585	0.261	No	
INC 25 µM (GS 51) vs Nil	5.533	1.664	3.585	0.801	No	
INC 25 µM (GS 61) vs Nil	4.233	1.273	3.585	0.948	No	
INC 50 µM (GS 61) vs Nil	3.100	0.932	3.585	0.993	No	
INC 50 µM (GS 65) vs Nil	2.042	0.614	3.585	1.000	No	
INC 25 µM (GS 39) vs Nil	2.000	0.602	3.585	1.000	No	
INC 10 µM (GS 65) vs Nil	1.375	0.414	3.585	1.000	No	
Tukey's d critical value:			5.071			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
INC 25 µM (GS 65)	67.533	2.351	62.594	72.472	A	
DMSO Control (GS 51, 61, 65, 65+15d)	62.767	2.351	57.828	67.706	A	B
INC 25 µM (GS 39, 51, 61, 65)	61.667	2.351	56.728	66.606	A	B
INC 25 µM (GS 51)	58.367	2.351	53.428	63.306	A	B
INC 25 µM (GS 61)	57.067	2.351	52.128	62.006	A	B
INC 50 µM (GS 61)	55.933	2.351	50.994	60.872	A	B
INC 50 µM (GS 65)	54.875	2.351	49.936	59.814		B
INC 25 µM (GS 39)	54.833	2.351	49.894	59.772		B
INC 10 µM (GS 65)	54.208	2.351	49.269	59.147		B
Nil	52.833	2.351	47.894	57.772		B

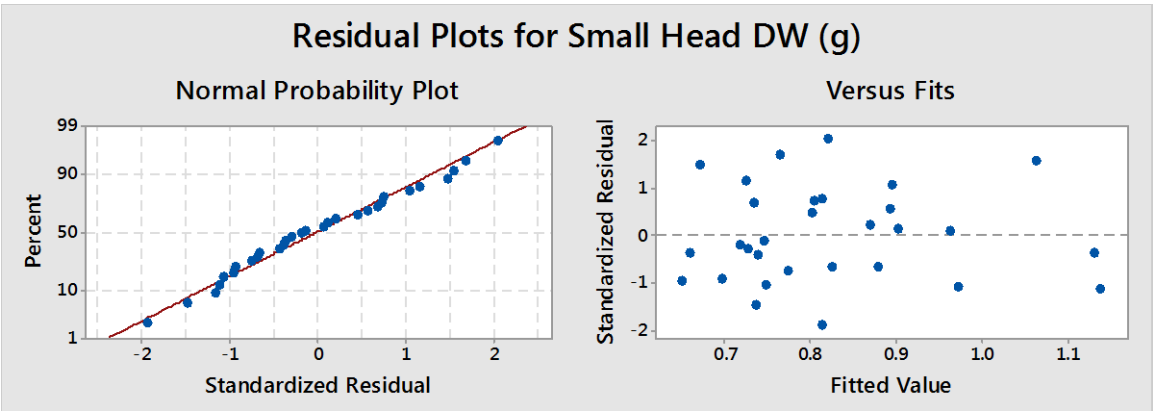
3.6.3 Quench barley 2014/15: Large Head DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.02115	0.01057	0.81	0.462
Treatment	9	0.15668	0.01741	1.33	0.290
Error	18	0.23592	0.01311		
Total	29	0.41374			

3.6.4 Quench barley 2014/15: Small Head DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.03505	0.017524	2.34	0.125
Treatment	9	0.44842	0.049825	6.66	**0.000**
Error	18	0.13459	0.007477		
Total	29	0.61806			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
INC 25 µM (GS 65) vs Nil	0.405	5.737	3.585	0.001	Yes	
INC 25 µM (GS 51) vs Nil	0.167	2.365	3.585	0.398	No	
INC 25 µM (GS 39, 51, 61, 65) vs Nil	0.144	2.041	3.585	0.585	No	
INC 25 µM (GS 61) vs Nil	0.087	1.233	3.585	0.956	No	
INC 25 µM (GS 39) vs Nil	0.078	1.112	3.585	0.977	No	
INC 10 µM (GS 65) vs Nil	0.039	0.554	3.585	1.000	No	
INC 50 µM (GS 65) vs Nil	0.014	0.193	3.585	1.000	No	
Nil vs INC 50 µM (GS 61)	0.009	0.132	3.585	1.000	No	
INC 25 µM (GS 65) vs DMSO Control (GS 51, 61, 65, 65+15d)	0.168	2.373	3.585	0.394	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 50 µM (GS 61)	0.247	3.495	3.585	0.060	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 50 µM (GS 65)	0.224	3.171	3.585	0.109	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 10 µM (GS 65)	0.198	2.809	3.585	0.205	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 25 µM (GS 39)	0.159	2.252	3.585	0.460	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 25 µM (GS 61)	0.150	2.130	3.585	0.531	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 25 µM (GS 39, 51, 61, 65)	0.093	1.323	3.585	0.935	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 25 µM (GS 51)	0.070	0.998	3.585	0.989	No	
Tukey's d critical value:			5.071			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
INC 25 µM (GS 65)	1.112	0.050	1.007	1.217	A	
DMSO Control (GS 51, 61, 65, 65+15d)	0.945	0.050	0.840	1.050	A	B
INC 25 µM (GS 51)	0.874	0.050	0.769	0.979	A	B
INC 25 µM (GS 39, 51, 61, 65)	0.851	0.050	0.746	0.956		B
INC 25 µM (GS 61)	0.794	0.050	0.689	0.899		B
INC 25 µM (GS 39)	0.786	0.050	0.681	0.891		B
INC 10 µM (GS 65)	0.746	0.050	0.641	0.851		B
INC 50 µM (GS 65)	0.721	0.050	0.616	0.826		B
Nil	0.707	0.050	0.602	0.812		B
INC 50 µM (GS 61)	0.698	0.050	0.593	0.803		B

3.6.5 Quench barley 2014/15: Tiller no.

Poisson Regression Analysis: Tiller no. versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	11	2.04867	41.74%	2.04867	0.18624	2.05	0.998
Block	2	0.03877	0.79%	0.03877	0.01938	0.04	0.981
Treatment	9	2.00990	40.95%	2.00990	0.22332	2.01	0.991
Error	18	2.85927	58.26%	2.85927	0.15885		
Total	29	4.90794	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value
Constant	1.780	0.261	(1.268, 2.293)	6.81
Block				
Block1	0.000000	0.000000	(0.000000, 0.000000)	*
Block2	-0.036	0.186	(-0.400, 0.328)	-0.20
Block3	-0.014	0.185	(-0.376, 0.348)	-0.07
Treatment				
DMSO Control (GS 51, 61, 65, 65+15d)	0.000000	0.000000	(0.000000, 0.000000)	*
INC 10 µM (GS 65)	-0.090	0.346	(-0.768, 0.588)	-0.26
INC 25 µM (GS 39)	-0.035	0.341	(-0.703, 0.634)	-0.10
INC 25 µM (GS 39, 51, 61, 65)	0.173	0.324	(-0.463, 0.809)	0.53
INC 25 µM (GS 51)	0.017	0.337	(-0.643, 0.677)	0.05
INC 25 µM (GS 61)	-0.216	0.358	(-0.917, 0.485)	-0.60
INC 25 µM (GS 65)	-0.006	0.339	(-0.669, 0.658)	-0.02
INC 50 µM (GS 61)	-0.071	0.344	(-0.746, 0.604)	-0.21
INC 50 µM (GS 65)	-0.035	0.341	(-0.703, 0.634)	-0.10
Nil	0.153	0.326	(-0.485, 0.792)	0.47

Term	*P*-Value	VIF
Constant	0.000	
Block		
Block1	*	*
Block2	0.845	1.32
Block3	0.941	1.32
Treatment		
DMSO Control (GS 51, 61, 65, 65+15d)	*	*
INC 10 µM (GS 65)	0.796	1.74
INC 25 µM (GS 39)	0.919	1.77
INC 25 µM (GS 39, 51, 61, 65)	0.594	1.93
INC 25 µM (GS 51)	0.960	1.81
INC 25 µM (GS 61)	0.546	1.66
INC 25 µM (GS 65)	0.986	1.79
INC 50 µM (GS 61)	0.837	1.75
INC 50 µM (GS 65)	0.919	1.77
Nil	0.638	1.91

Regression Equation

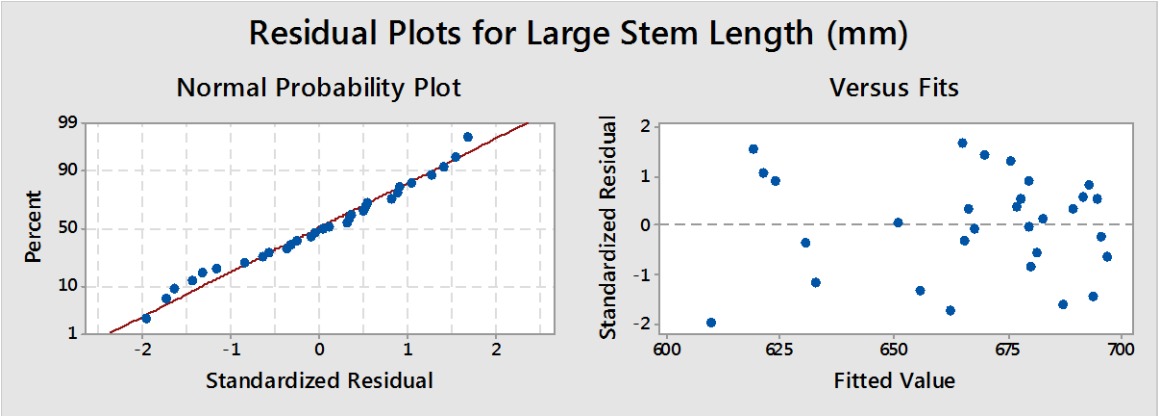
Tiller no. = exp(Y')

Y' = 1.780 +0.0Block_Block1 -0.036Block_Block2 -0.014Block_Block3
+0.0Treatment_DMSO Control (GS 51, 61, 65, 65+15d) -0.090Treatment_INC 10 µM (GS 65)
-0.035Treatment_INC 25 µM (GS 39) +0.173Treatment_INC 25 µM (GS 39, 51, 61, 65)
+0.017Treatment_INC 25 µM (GS 51) -0.216Treatment_INC 25 µM (GS 61)
-0.006Treatment_INC 25 µM (GS 65) -0.071Treatment_INC 50 µM (GS 61)
-0.035Treatment_INC 50 µM (GS 65) +0.153Treatment_Nil

Goodness-o*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	18	3.91543	0.21752	3.92	1.000
Pearson	18	3.80115	0.21118	3.80	1.000

3.6.6 Quench barley 2014/15: Large Stem Length

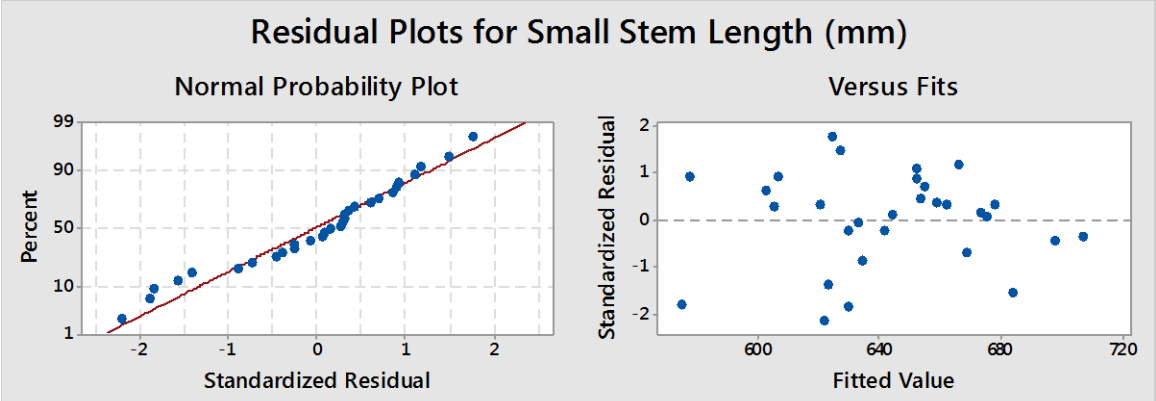


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	1136	568.0	0.81	0.462
Treatment	9	18004	2000.5	2.84	**0.028**
Error	18	12683	704.6		
Total	29	31823			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:					
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
INC 10 µM (GS 65) vs DMSO Control (GS 51, 61, 65, 65+15d)	32.033	1.478	3.585	0.884	No
INC 25 µM (GS 61) vs DMSO Control (GS 51, 61, 65, 65+15d)	28.900	1.333	3.585	0.932	No
INC 25 µM (GS 39) vs DMSO Control (GS 51, 61, 65, 65+15d)	24.600	1.135	3.585	0.974	No
INC 50 µM (GS 61) vs DMSO Control (GS 51, 61, 65, 65+15d)	15.333	0.707	3.585	0.999	No
INC 25 µM (GS 65) vs DMSO Control (GS 51, 61, 65, 65+15d)	14.500	0.669	3.585	0.999	No
INC 25 µM (GS 51) vs DMSO Control (GS 51, 61, 65, 65+15d)	4.900	0.226	3.585	1.000	No
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 50 µM (GS 65)	41.267	1.904	3.585	0.667	No
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 25 µM (GS 39, 51, 61, 65)	32.133	1.483	3.585	0.882	No
INC 10 µM (GS 65) vs Nil	1.570	0.072	3.585	1.000	No
Nil vs INC 50 µM (GS 65)	71.730	3.310	3.585	0.085	No
Nil vs INC 25 µM (GS 39, 51, 61, 65)	62.596	2.888	3.585	0.179	No
Nil vs INC 25 µM (GS 51)	25.563	1.179	3.585	0.967	No
Nil vs INC 25 µM (GS 65)	15.963	0.737	3.585	0.999	No
Nil vs INC 50 µM (GS 61)	15.130	0.698	3.585	0.999	No
Nil vs INC 25 µM (GS 39)	5.863	0.271	3.585	1.000	No
Nil vs INC 25 µM (GS 61)	1.563	0.072	3.585	1.000	No
Tukey's d critical value:			5.071		
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
INC 10 µM (GS 65)	691.633	15.325	659.436	723.831	A
Nil	690.063	15.325	657.865	722.261	A
INC 25 µM (GS 61)	688.500	15.325	656.302	720.698	A
INC 25 µM (GS 39)	684.200	15.325	652.002	716.398	A
INC 50 µM (GS 61)	674.933	15.325	642.736	707.131	A
INC 25 µM (GS 65)	674.100	15.325	641.902	706.298	A
INC 25 µM (GS 51)	664.500	15.325	632.302	696.698	A
DMSO Control (GS 51, 61, 65, 65+15d)	659.600	15.325	627.402	691.798	A
INC 25 µM (GS 39, 51, 61, 65)	627.467	15.325	595.269	659.664	A
INC 50 µM (GS 65)	618.333	15.325	586.136	650.531	A

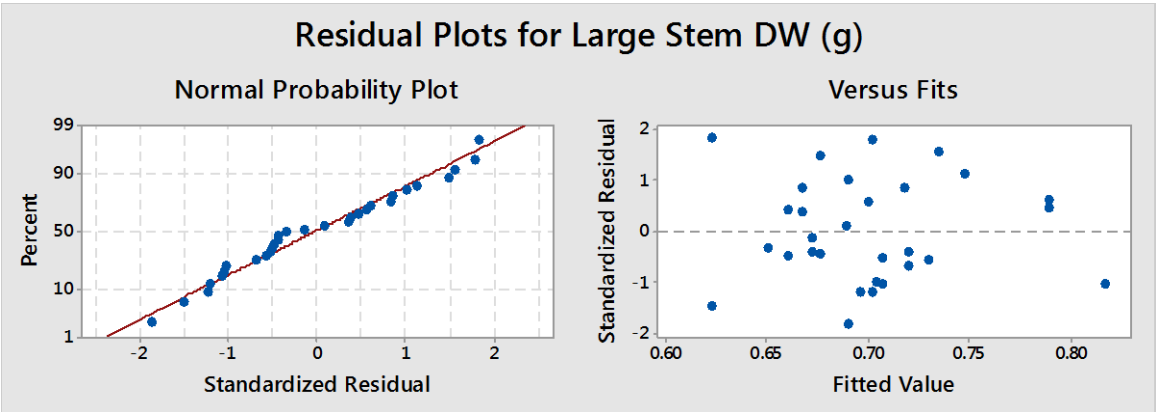
3.6.7 Quench barley 2014/15: Small Stem Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	6288	3144	1.62	0.226
Treatment	9	23746	2638	1.36	0.278
Error	18	35025	1946		
Total	29	65058			

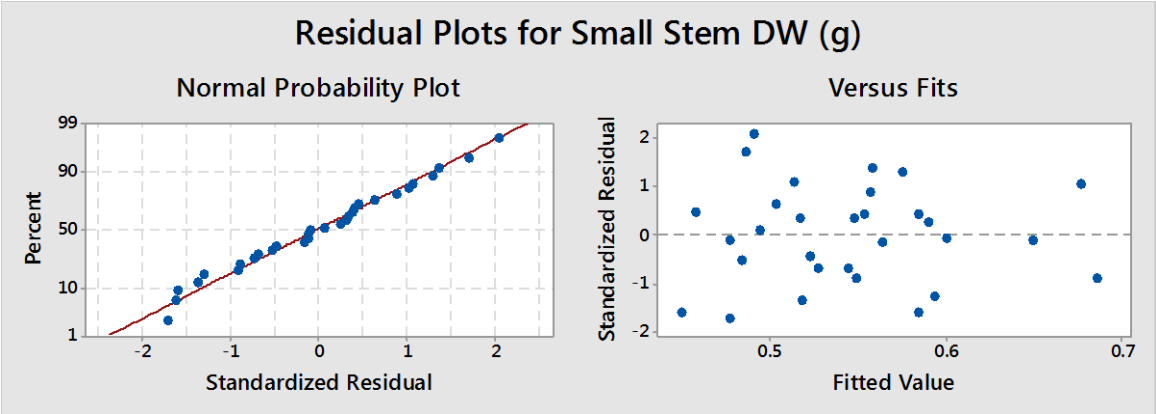
3.6.8 Quench barley 2014/15: Large Stem DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.005326	0.002663	0.59	0.567
Treatment	9	0.052843	0.005871	1.29	0.306
Error	18	0.081788	0.004544		
Total	29	0.139956			

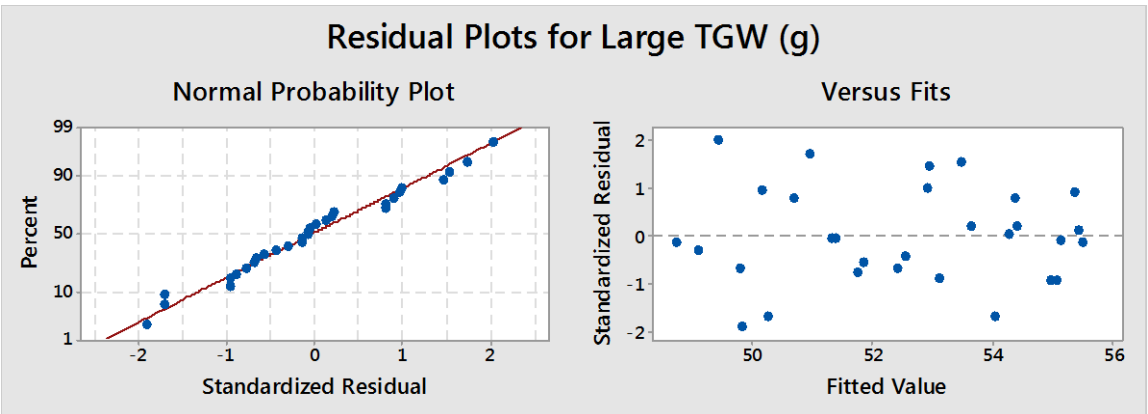
3.6.9 Quench barley 2014/15: Small Stem DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.007137	0.003569	0.75	0.485
Treatment	9	0.096957	0.010773	2.27	0.066
Error	18	0.085267	0.004737		
Total	29	0.189362			

3.6.10 Quench barley 2014/15: Large TGW



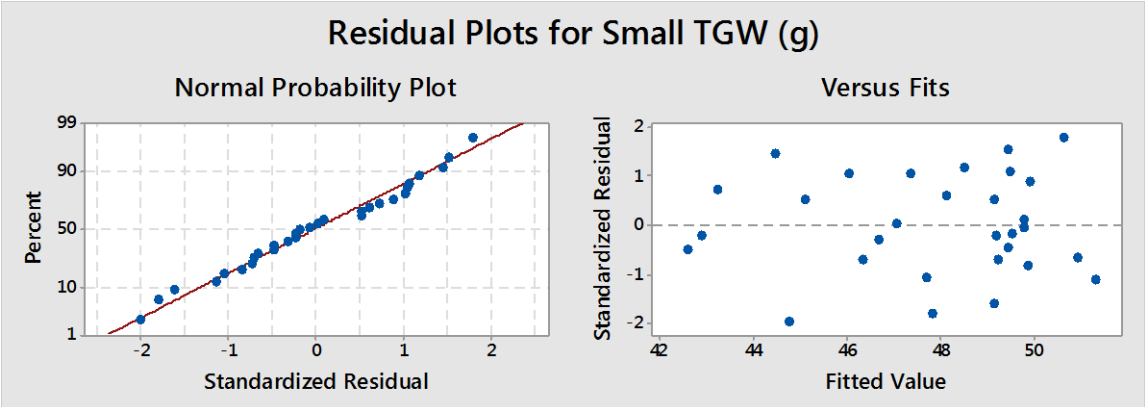
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	5.915	2.957	1.35	0.284
Treatment	9	124.430	13.826	6.31	**0.000**
Error	18	39.460	2.192		
Total	29	169.804			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
Nil vs INC 25 µM (GS 39, 51, 61, 65)	5.678	4.697	3.585	0.005	Yes	
Nil vs INC 25 µM (GS 65)	5.314	4.396	3.585	0.010	Yes	
Nil vs INC 50 µM (GS 61)	4.167	3.447	3.585	0.065	No	
Nil vs INC 25 µM (GS 61)	3.731	3.086	3.585	0.127	No	
Nil vs INC 25 µM (GS 51)	2.009	1.662	3.585	0.802	No	
Nil vs INC 25 µM (GS 39)	1.466	1.212	3.585	0.961	No	
Nil vs INC 50 µM (GS 65)	0.133	0.110	3.585	1.000	No	
Nil vs INC 10 µM (GS 65)	0.051	0.042	3.585	1.000	No	
INC 10 µM (GS 65) vs DMSO Control (GS 51, 61, 65, 65+15d)	2.526	2.089	3.585	0.556	No	
INC 50 µM (GS 65) vs DMSO Control (GS 51, 61, 65, 65+15d)	2.443	2.021	3.585	0.597	No	
INC 25 µM (GS 39) vs DMSO Control (GS 51, 61, 65, 65+15d)	1.111	0.919	3.585	0.994	No	
INC 25 µM (GS 51) vs DMSO Control (GS 51, 61, 65, 65+15d)	0.568	0.470	3.585	1.000	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 25 µM (GS 39, 51, 61, 65)	3.101	2.565	3.585	0.300	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 25 µM (GS 65)	2.738	2.265	3.585	0.453	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 50 µM (GS 61)	1.590	1.315	3.585	0.937	No	
DMSO Control (GS 51, 61, 65, 65+15d) vs INC 25 µM (GS 61)	1.154	0.955	3.585	0.992	No	
Tukey's d critical value:			5.071			

Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
Nil	55.014	0.855	53.219	56.810	A	
INC 10 µM (GS 65)	54.963	0.855	53.167	56.759	A	
INC 50 µM (GS 65)	54.881	0.855	53.085	56.677	A	
INC 25 µM (GS 39)	53.549	0.855	51.753	55.345	A	B
INC 25 µM (GS 51)	53.006	0.855	51.210	54.801	A	B
DMSO Control (GS 51, 61, 65, 65+15d)	52.438	0.855	50.642	54.234	A	B
INC 25 µM (GS 61)	51.283	0.855	49.487	53.079	A	B
INC 50 µM (GS 61)	50.848	0.855	49.052	52.644	A	B
INC 25 µM (GS 65)	49.700	0.855	47.904	51.496		B
INC 25 µM (GS 39, 51, 61, 65)	49.337	0.855	47.541	51.133		B

3.6.11 Quench barley 2014/15: Small TGW



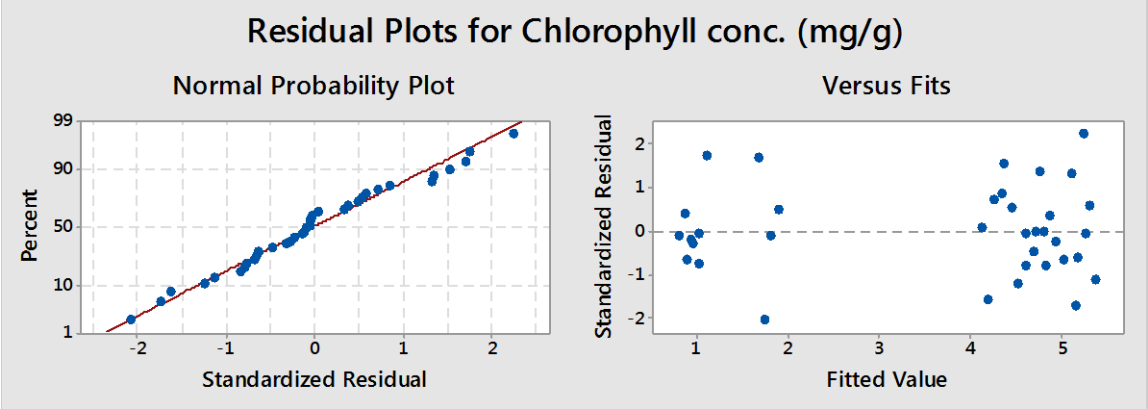
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	2.225	1.112	0.41	0.668
Treatment	9	173.153	19.239	7.13	**0.000**
Error	18	48.587	2.699		
Total	29	223.966			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:							
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
INC 25 µM (GS 61)	51.006	0.949	49.013	52.998	A		
DMSO Control (GS 51, 61, 65, 65+15d)	49.588	0.949	47.595	51.581	A		
INC 25 µM (GS 39)	49.567	0.949	47.574	51.560	A	B	
INC 25 µM (GS 65)	49.494	0.949	47.502	51.487	A	B	
INC 25 µM (GS 51)	49.492	0.949	47.499	51.485	A	B	
Nil	48.177	0.949	46.184	50.170	A	B	
INC 10 µM (GS 65)	47.401	0.949	45.408	49.394	A	B	C
INC 50 µM (GS 61)	46.372	0.949	44.379	48.365	A	B	C
INC 25 µM (GS 39, 51, 61, 65)	44.778	0.949	42.785	46.771		B	C
INC 50 µM (GS 65)	42.903	0.949	40.910	44.896			C

3.7 Tavern wheat 2014/15 chlorophyll

3.7.1 Tavern wheat 2014/15: Flag leaf

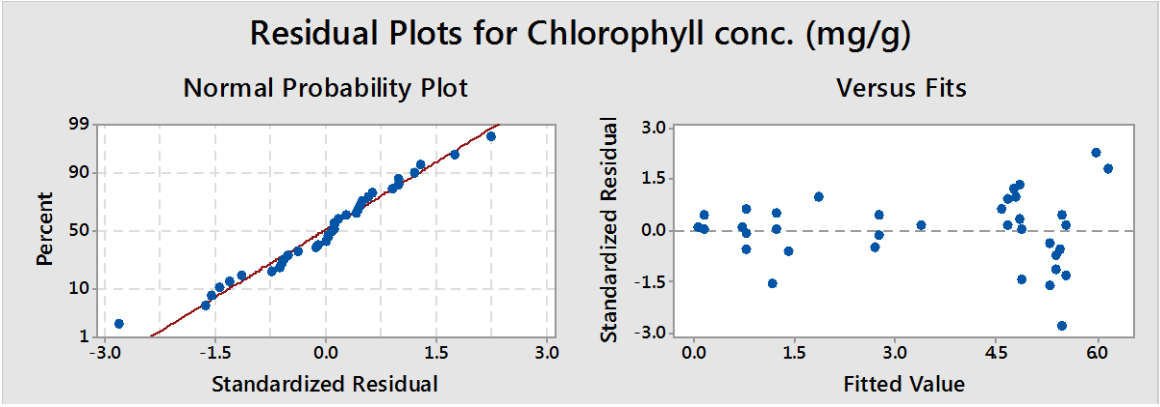


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	0.241	0.0802	0.12	0.948
Time	2	100.491	50.2453	75.08	0.000
Treatment	2	1.532	0.7662	1.14	0.335
Time*Treatment	4	3.349	0.8371	1.25	0.316
Error	24	16.062	0.6693		
Total	35	121.675			

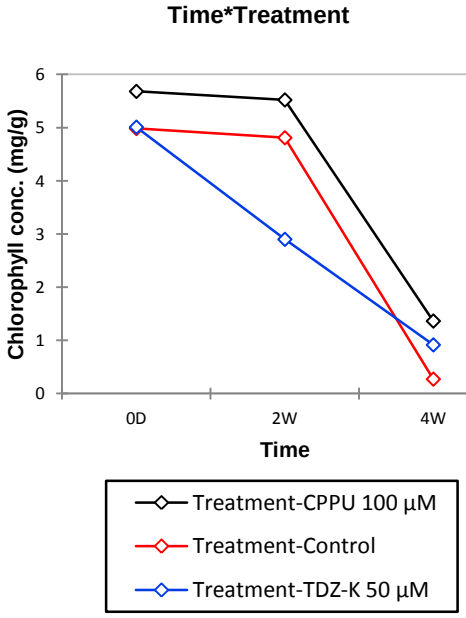
Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):						
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
0D	4.825	0.236	4.338	5.313	A	
2W	4.734	0.236	4.247	5.221	A	
4W	1.236	0.236	0.749	1.724		B
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
CPPU 100 µM	3.886	0.236	3.398	4.373	A	
TDZ-K 50 µM	3.501	0.236	3.014	3.988	A	
Control	3.409	0.236	2.922	3.897	A	
Category	LS means(Chlorophyll conc. (mg/g))	Groups				
0D*TDZ-K 50 µM	5.269	A				
2W*CPPU 100 µM	5.141	A				
2W*Control	4.830	A				
0D*CPPU 100 µM	4.722	A				
0D*Control	4.486	A				
2W*TDZ-K 50 µM	4.232	A				
4W*CPPU 100 µM	1.795		B			
4W*TDZ-K 50 µM	1.002		B			
4W*Control	0.912		B			

3.7.2 Tavern wheat 2014/15: Primary leaf



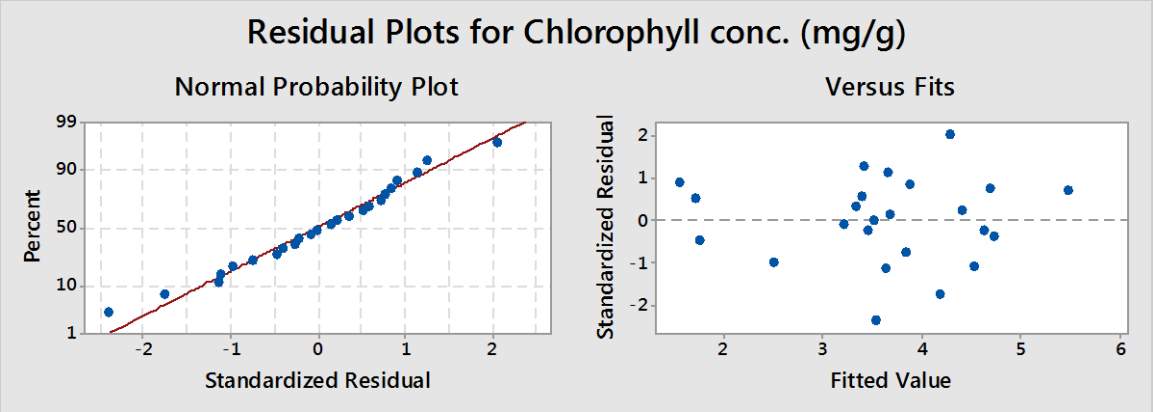
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	2.941	0.9805	1.88	0.161
Time leaf acquired	2	129.835	64.9175	124.22	0.000
Treatment	2	9.682	4.8411	9.26	0.001
Time leaf acquired*Treatment	4	8.668	2.1671	4.15	0.011
Error	24	12.543	0.5226		
Total	35	163.669			



Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):							
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
0D	5.225	0.209	4.794	5.656	A		
2W	4.410	0.209	3.980	4.841		B	
4W	0.851	0.209	0.421	1.282			C
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
CPPU 100 µM	4.189	0.209	3.758	4.620	A		
Control	3.355	0.209	2.925	3.786		B	
TDZ-K 50 µM	2.942	0.209	2.512	3.373		B	
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Time-0D*Treatment-CPPU 100 µM	5.682	0.361	4.936	6.428	A		
Time-2W*Treatment-CPPU 100 µM	5.521	0.361	4.775	6.267	A		
Time-0D*Treatment-TDZ-K 50 µM	5.010	0.361	4.264	5.756	A		
Time-0D*Treatment-Control	4.984	0.361	4.238	5.730	A		
Time-2W*Treatment-Control	4.809	0.361	4.063	5.555	A		
Time-2W*Treatment-TDZ-K 50 µM	2.901	0.361	2.155	3.647		B	
Time-4W*Treatment-CPPU 100 µM	1.365	0.361	0.619	2.111		B	C
Time-4W*Treatment-TDZ-K 50 µM	0.916	0.361	0.170	1.662			C
Time-4W*Treatment-Control	0.273	0.361	-0.473	1.019			C

3.7.3 Tavern wheat 2014/15: Secondary leaf



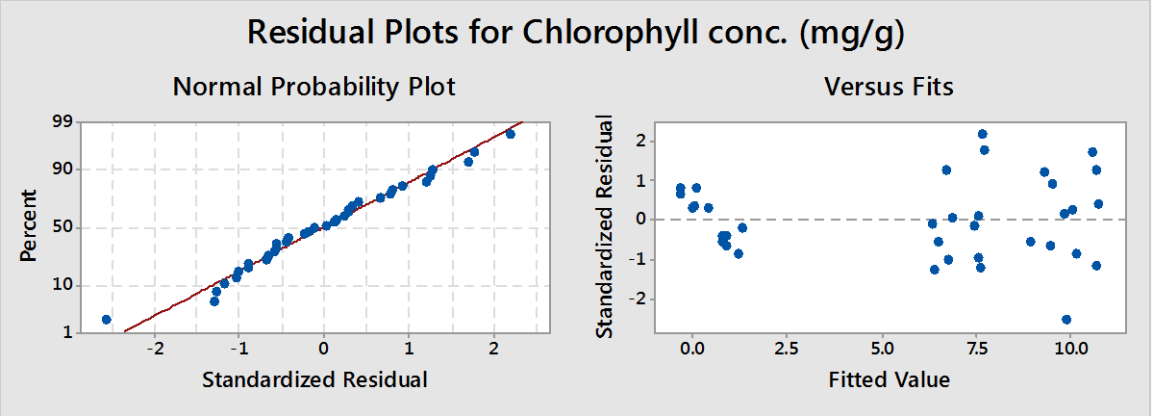
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	3.247	1.0822	1.30	0.312
Time leaf acquired	1	6.305	6.3051	7.56	0.015
Treatment	2	10.512	5.2562	6.30	0.010
Time leaf acquired*Treatment	2	2.234	1.1170	1.34	0.292
Error	15	12.509	0.8340		
Total	23	34.808			

Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):						
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
0D	4.145	0.264	3.583	4.707	A	
2W	3.120	0.264	2.558	3.682		B
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
CPPU 100 µM	4.271	0.323	3.583	4.959	A	
Control	3.907	0.323	3.219	4.595	A	
TDZ-K 50 µM	2.721	0.323	2.032	3.409		B
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
Time-0D*Treatment-CPPU 100 µM	4.863	0.457	3.890	5.837	A	
Time-0D*Treatment-Control	4.012	0.457	3.039	4.985	A	
Time-2W*Treatment-Control	3.801	0.457	2.828	4.775	A	B
Time-2W*Treatment-CPPU 100 µM	3.678	0.457	2.705	4.651	A	B
Time-0D*Treatment-TDZ-K 50 µM	3.560	0.457	2.587	4.534	A	B
Time-2W*Treatment-TDZ-K 50 µM	1.881	0.457	0.908	2.854		B

3.8 Quench barley 2014/15 chlorophyll

3.8.1 Quench barley 2014/15: Flag

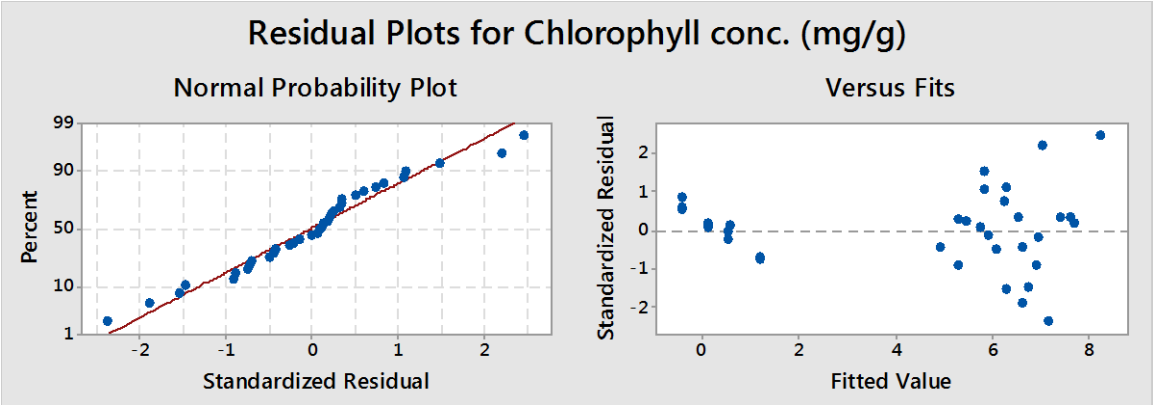


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	9.548	3.183	2.37	0.096
Time leaf acquired	2	572.357	286.179	212.73	0.000
Treatment	2	0.828	0.414	0.31	0.738
Time leaf acquired*Treatment	4	0.578	0.145	0.11	0.979
Error	24	32.286	1.345		
Total	35	615.597			

Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):							
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
2.5W	9.996	0.335	9.305	10.687	A		
0D	7.103	0.335	6.412	7.794		B	
5.5W	0.471	0.335	-0.220	1.162			C
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
INCYDE 25 µM	6.030	0.335	5.338	6.721	A		
Control	5.880	0.335	5.189	6.571	A		
INCYDE 50 µM	5.660	0.335	4.969	6.351	A		
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Time leaf acquired-2.5W*Treatment-Control	10.221	0.580	9.024	11.417	A		
Time leaf acquired-2.5W*Treatment-INCYDE 25 µM	10.154	0.580	8.957	11.351	A		
Time leaf acquired-2.5W*Treatment-INCYDE 50 µM	9.615	0.580	8.418	10.812	A	B	
Time leaf acquired-0D*Treatment-INCYDE 25 µM	7.182	0.580	5.985	8.379		B	
Time leaf acquired-0D*Treatment-Control	7.098	0.580	5.901	8.295		B	
Time leaf acquired-0D*Treatment-INCYDE 50 µM	7.027	0.580	5.831	8.224		B	
Time leaf acquired-5.5W*Treatment-INCYDE 25 µM	0.752	0.580	-0.445	1.949			C
Time leaf acquired-5.5W*Treatment-INCYDE 50 µM	0.339	0.580	-0.858	1.536			C
Time leaf acquired-5.5W*Treatment-Control	0.322	0.580	-0.875	1.519			C

3.8.2 Quench barley 2014/15: Primary

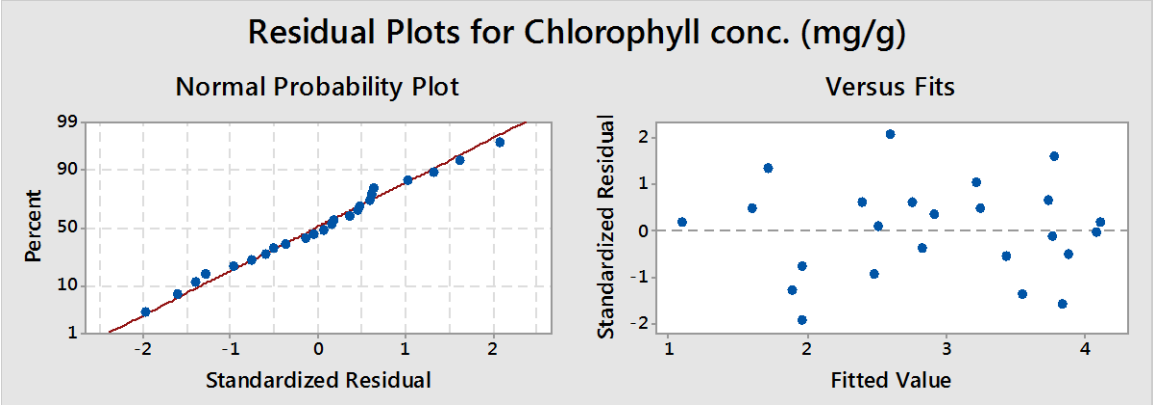


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	12.857	4.286	2.07	0.131
Time leaf acquired	2	299.439	149.720	72.21	0.000
Treatment	2	1.094	0.547	0.26	0.770
Time leaf acquired*Treatment	4	3.977	0.994	0.48	0.750
Error	24	49.764	2.073		
Total	35	367.131			

Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):						
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
2.5W	6.796	0.416	5.938	7.654	A	
0D	6.135	0.416	5.277	6.993	A	
5.5W	0.374	0.416	-0.483	1.232		B
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
INCYDE 25 µM	4.603	0.416	3.745	5.461	A	
Control	4.508	0.416	3.650	5.366	A	
INCYDE 50 µM	4.195	0.416	3.337	5.053	A	
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
Time leaf acquired-2.5W*Treatment-Control	7.430	0.720	5.944	8.916	A	
Time leaf acquired-2.5W*Treatment-INCYDE 25 µM	6.873	0.720	5.387	8.359	A	
Time leaf acquired-0D*Treatment-INCYDE 25 µM	6.566	0.720	5.080	8.052	A	
Time leaf acquired-0D*Treatment-INCYDE 50 µM	6.113	0.720	4.627	7.599	A	
Time leaf acquired-2.5W*Treatment-INCYDE 50 µM	6.085	0.720	4.599	7.571	A	
Time leaf acquired-0D*Treatment-Control	5.726	0.720	4.240	7.212	A	
Time leaf acquired-5.5W*Treatment-INCYDE 50 µM	0.387	0.720	-1.099	1.873		B
Time leaf acquired-5.5W*Treatment-INCYDE 25 µM	0.369	0.720	-1.117	1.855		B
Time leaf acquired-5.5W*Treatment-Control	0.368	0.720	-1.118	1.854		B

3.8.3 Quench barley 2014/15: Secondary

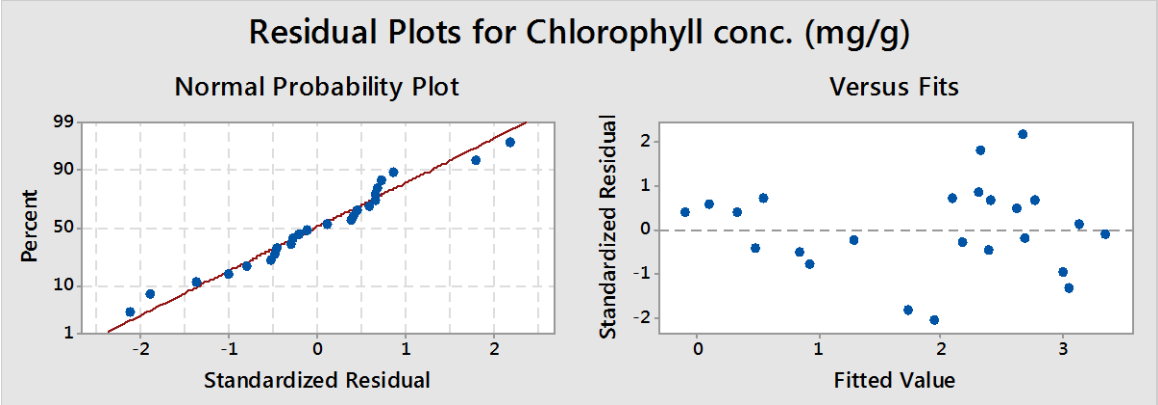


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	2.4223	0.8074	3.24	0.052
Time leaf acquired	1	13.2794	13.2794	53.27	0.000
Treatment	2	0.8414	0.4207	1.69	0.218
Time leaf acquired*Treatment	2	1.3248	0.6624	2.66	0.103
Error	15	3.7391	0.2493		
Total	23	21.6071			

Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):							
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
0D	3.639	0.144	3.331	3.946	A		
2.5W	2.151	0.144	1.844	2.458		B	
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Control	3.117	0.177	2.741	3.494	A		
INCYDE 50 µM	2.908	0.177	2.532	3.284	A		
INCYDE 25 µM	2.659	0.177	2.283	3.035	A		
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Time leaf acquired-0D*Treatment-Control	3.763	0.250	3.231	4.295	A		
Time leaf acquired-0D*Treatment-INCYDE 25 µM	3.727	0.250	3.195	4.259	A		
Time leaf acquired-0D*Treatment-INCYDE 50 µM	3.426	0.250	2.894	3.958	A	B	
Time leaf acquired-2.5W*Treatment-Control	2.471	0.250	1.939	3.003		B	C
Time leaf acquired-2.5W*Treatment-INCYDE 50 µM	2.390	0.250	1.858	2.922		B	C
Time leaf acquired-2.5W*Treatment-INCYDE 25 µM	1.591	0.250	1.059	2.124			C

3.8.4 Quench barley 2014/15: Tertiary



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	3.160	1.0534	1.67	0.216
Time leaf acquired	1	15.148	15.1477	24.04	0.000
Treatment	2	3.253	1.6264	2.58	0.109
Time leaf acquired*Treatment	2	4.447	2.2237	3.53	0.055
Error	15	9.451	0.6300		
Total	23	35.459			

Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):							
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
0D	2.678	0.229	2.189	3.166	A		
2.5W	1.089	0.229	0.600	1.577		B	
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
INCYDE 50 µM	2.402	0.281	1.804	3.001	A		
Control	1.657	0.281	1.059	2.255	A		
INCYDE 25 µM	1.590	0.281	0.992	2.188	A		
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Time leaf acquired-0D*Treatment-INCYDE 25 µM	2.858	0.397	2.012	3.704	A		
Time leaf acquired-0D*Treatment-INCYDE 50 µM	2.629	0.397	1.783	3.475	A		
Time leaf acquired-0D*Treatment-Control	2.546	0.397	1.700	3.392	A	B	
Time leaf acquired-2.5W*Treatment-INCYDE 50 µM	2.176	0.397	1.330	3.022	A	B	
Time leaf acquired-2.5W*Treatment-Control	0.768	0.397	-0.077	1.614		B	C
Time leaf acquired-2.5W*Treatment-INCYDE 25 µM	0.322	0.397	-0.524	1.168			C

Appendix 4: Chapter 4 statistical analyses for wheat and barley pot trials

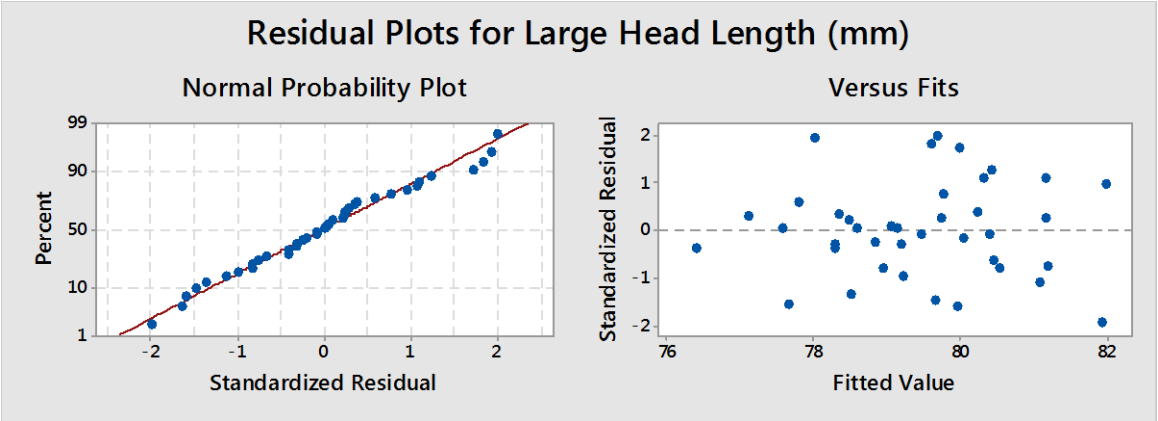
4.1 Summary of application rates for pot trials

Trial, cultivar and season	**Wheat Trial 1 cv. Morph (2013/14)**	
Sowing date	December 2013	
Harvest date	May 2014	
Sowing rate	60.7 kg/ha (132 seeds/m²)	
Sowing depth	3-4 cm	
Nitrogen application	289.5 kgN/ha (split over 154.4 kgN/ha at sowing, 77.2 kgN/ha at stem elongation, 57.9 kgN/ha following anthesis) with Tui Novatec Premium Fertiliser (Tui Garden) fertiliser	
Irrigation	Irrigation once every 1-3 days	
Fungicide	Yates Fungus Fighter (Yates) applied at 10 mL/L. Note: Fungal growth was an issue due to a significant period of rain.	
Insecticide	Yates Pyrethrum Insecticide (Yates) applied at 5 mL/L	
Trial, cultivar and season	**Wheat Trial 2A cv. Morph (2014/15)**	**Barley Trial 2B cv. Tavern (2014/15)**
Sowing date	Late November 2014	Late November 2014
Harvest date	April-May 2015	April-May 2015
Sowing rate	60.7 kg/ha (132 seeds/m²)	63.3 kg/ha (132 seeds/m²)
Sowing depth	3-4 cm	3-4 cm
Nitrogen application	69.5 kgN/ha (split over 23.2 kgN/ha at sowing, 46.3 kgN/ha at tillering) with Tui Novatec Premium Fertiliser (Tui Garden) fertiliser	55.6 kgN/ha (split over 11.6 kgN/ha at sowing, 15.4 kgN/ha at tillering, 28.6 kgN/ha at stem elongation with Tui Novatec Premium Fertiliser (Tui Garden) fertiliser
Irrigation	Irrigation once every 1-3 days	Irrigation once every 1-3 days
Fungicide	Yates Fungus Fighter (Yates) applied at 10 mL/L	Yates Fungus Fighter (Yates) applied at 10 mL/L
Insecticide	Yates Pyrethrum Insecticide (Yates) applied at 5 mL/L	Yates Pyrethrum Insecticide (Yates) applied at 5 mL/L
Trial, cultivar and season	**Wheat Drought Trial 3A cv. Morph (2014/15)**	**Barley Drought Trial 3B cv. Tavern (2014/15)**
Sowing date	Late December 2014	Late December 2014
Harvest date	May 2015	May 2015
Sowing rate	48.3 kg/ha (105 seeds/m²)	50.4 kg/ha (105 seeds/m²)
Sowing depth	3 cm	3 cm
Nitrogen application	69 kgN/ha (split over 23 kgN/ha at sowing, 46 kgN/ha at tillering) with Tui Novatech Premium (Tui Garden) fertiliser	57 kgN/ha (split over 13.5 kgN/ha at sowing, 14.3 kgN/ha at tillering, 29 kgN/ha) with Tui Novatech Premium (Tui Garden) fertiliser
Irrigation	Irrigation once every 2 days	Irrigation once every 2 days
Fungicide	None	Yates Fungus Fighter (Yates) applied at 10 mL/L
Insecticide	Yates Pyrethrum Insecticide (Yates) applied at 5 mL/L	None

Trial, cultivar and season	**Wheat Trial 4A cv. Morph (2015/16)**	**Barley Trial 4B cv. Fairview (2015/16)**
Sowing date	Late October 2015	Late October 2015
Harvest date	March 2016	March 2016
Sowing rate	96.6 kg/ha (210 seeds/m²)	103 kg/ha (210 seeds/m²)
Sowing depth	3 cm	3 cm
Nitrogen application	250 kgN/ha (split over 100 kgN/ha early in leaf development and 150 kgN/ha at tillering) with Tui Novatech Premium (Tui) fertiliser	220 kgN/ha (split over 100 kgN/ha at tillering and 120 kgN/ha during stem elongation) with Tui Novatech Premium (Tui) fertiliser
Irrigation	Regular irrigation once every 1-3 days based on demand.	Regular irrigation once every 1-3 days based on demand.

4.2 Trial 1 wheat

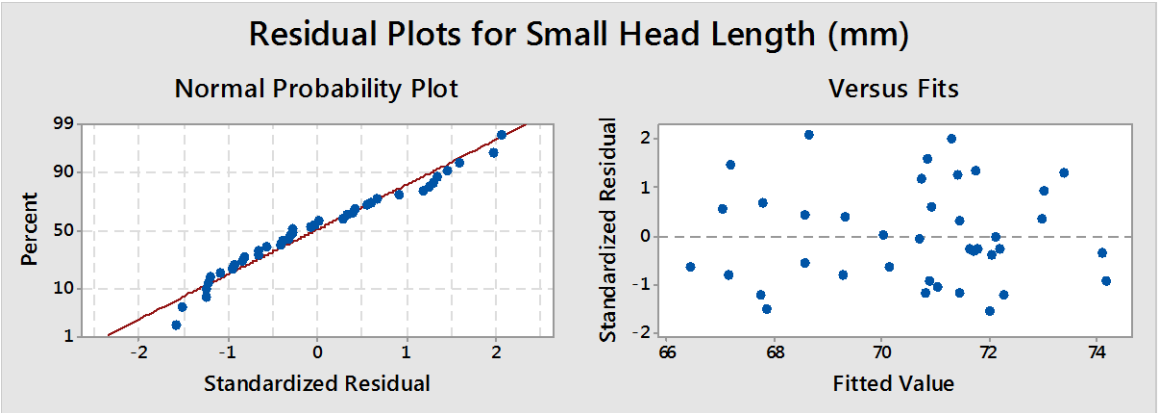
4.2.1 Trial 1 wheat: Large Head Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	24.88	12.442	1.52	0.239
Treatment	12	39.57	3.298	0.40	0.948
Error	24	196.55	8.190		
Total	38	261.01			

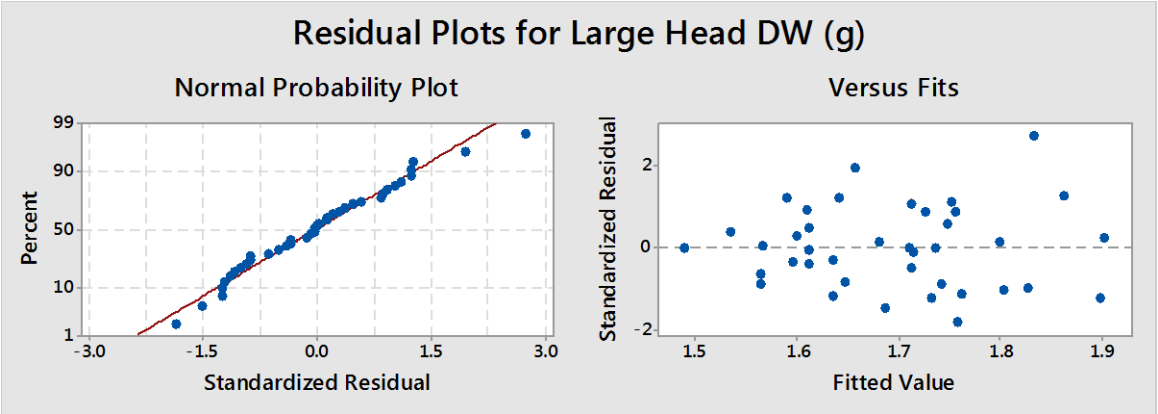
4.2.2 Trial 1 wheat: Small Head Length



Analysis of Variance

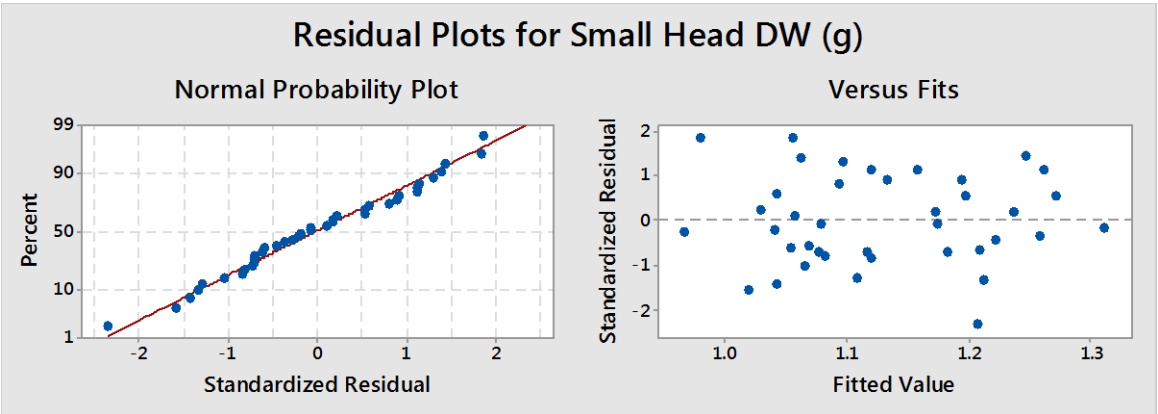
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	4.638	2.319	0.39	0.678
Treatment	12	152.774	12.731	2.17	0.052
Error	24	141.044	5.877		
Total	38	298.456			

4.2.3 Trial 1 wheat: Large Head DW



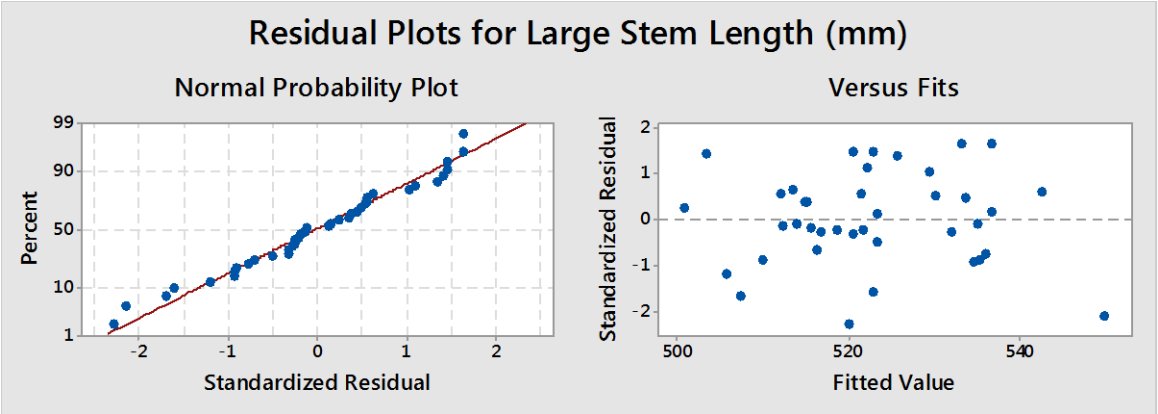
Analysis of Variance					
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.1475	0.07374	5.08	0.014
Treatment	12	0.2428	0.02023	1.39	0.235
Error	24	0.3482	0.01451		
Total	38	0.7385			

4.2.4 Trial 1 wheat: Small Head DW



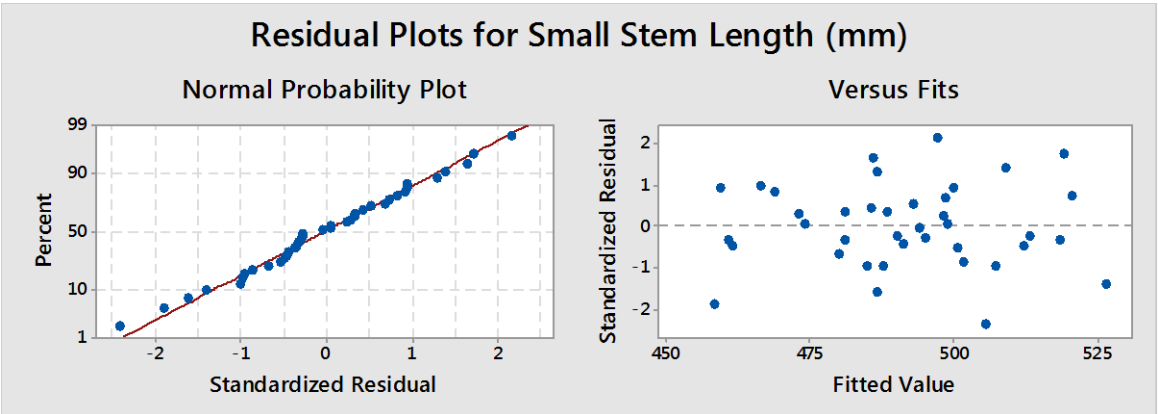
Analysis of Variance					
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.02014	0.01007	0.77	0.475
Treatment	12	0.27055	0.02255	1.72	0.125
Error	24	0.31471	0.01311		
Total	38	0.60540			

4.2.5 Trial 1 wheat: Large Stem Length



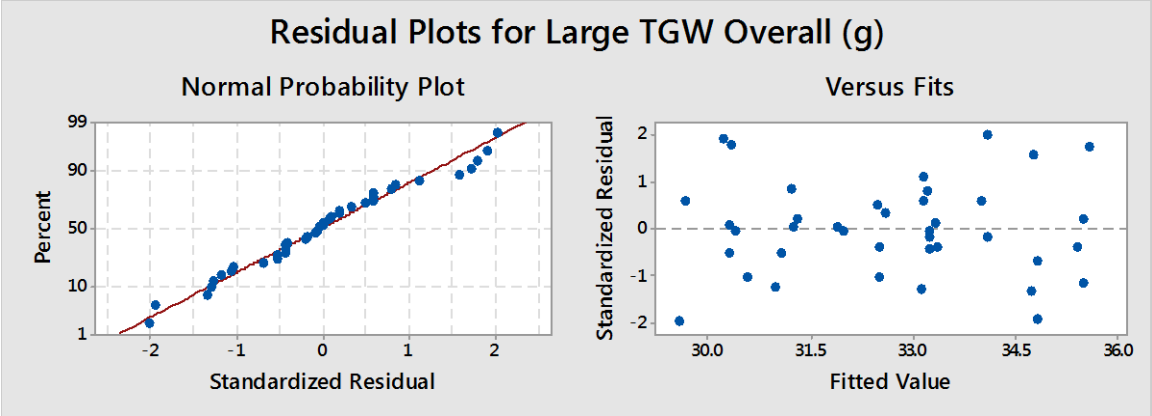
Analysis of Variance					
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	2666	1333.0	5.16	0.014
Treatment	12	2154	179.5	0.69	0.741
Error	24	6202	258.4		
Total	38	11023			

4.2.6 Trial 1 wheat: Small Stem Length



Analysis of Variance					
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	522.7	261.3	0.52	0.603
Treatment	12	11471.7	956.0	1.89	0.089
Error	24	12125.9	505.2		
Total	38	24120.3			

4.2.7 Trial 1 wheat: Large TGW Overall



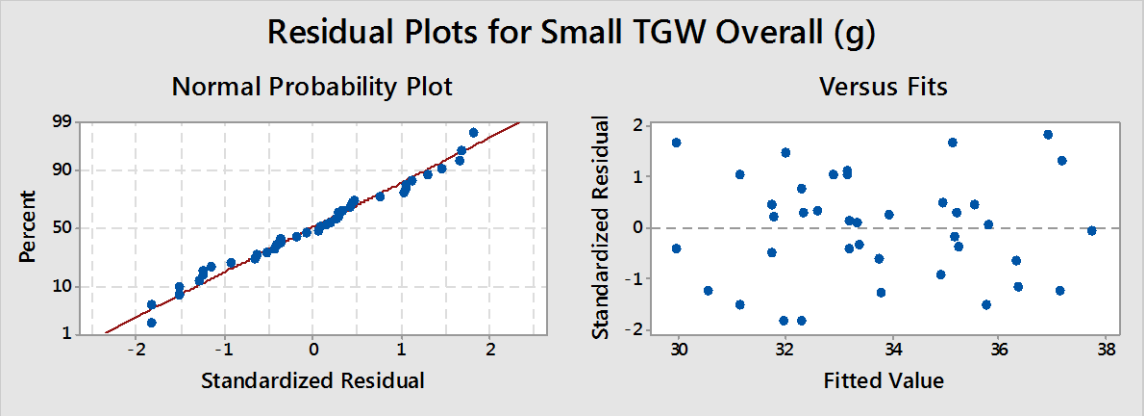
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	4.219	2.109	0.65	0.529
Treatment	12	117.815	9.818	3.04	0.010
Error	24	77.428	3.226		
Total	38	199.462			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Large TGW Overall (g)):					
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
INC 25 (GS 60) vs Nil	2.329	1.588	3.662	0.917	No
INC 25 (GS 60) vs DMSO Control (GS 39, 51, 60, 65)	2.244	1.530	3.662	0.934	No
INC 25 (GS 60) vs DMSO Control (GS 60, 65+2W)	0.072	0.049	3.662	1.000	No
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 65)	5.190	3.539	3.662	0.065	No
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 39)	5.101	3.478	3.662	0.074	No
DMSO Control (GS 60, 65+2W) vs INC 10 (GS 65)	4.441	3.028	3.662	0.178	No
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 39, 51, 60, 65)	4.197	2.862	3.662	0.238	No
DMSO Control (GS 60, 65+2W) vs INC 50 (GS 60)	3.523	2.402	3.662	0.474	No
DMSO Control (GS 60, 65+2W) vs TDZ-K 25 (GS 60+65+2W)	2.304	1.571	3.662	0.922	No
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 51)	2.263	1.543	3.662	0.931	No
DMSO Control (GS 60, 65+2W) vs INC 50 (GS 65)	1.405	0.958	3.662	0.998	No
DMSO Control (GS 60, 65+2W) vs TDZ-K 10 (GS 60, 65+2W)	0.676	0.461	3.662	1.000	No
TDZ-K 10 (GS 60, 65+2W) vs Nil	1.581	1.078	3.662	0.995	No
TDZ-K 10 (GS 60, 65+2W) vs DMSO Control (GS 39, 51, 60, 65)	1.497	1.021	3.662	0.997	No
INC 50 (GS 65) vs Nil	0.852	0.581	3.662	1.000	No
INC 50 (GS 65) vs DMSO Control (GS 39, 51, 60, 65)	0.768	0.523	3.662	1.000	No
DMSO Control (GS 39, 51, 60, 65) vs INC 25 (GS 65)	3.018	2.058	3.662	0.689	No
DMSO Control (GS 39, 51, 60, 65) vs INC 25 (GS 39)	2.928	1.997	3.662	0.725	No
DMSO Control (GS 39, 51, 60, 65) vs INC 10 (GS 65)	2.268	1.547	3.662	0.930	No
DMSO Control (GS 39, 51, 60, 65) vs INC 25 (GS 39, 51, 60, 65)	2.024	1.380	3.662	0.968	No
DMSO Control (GS 39, 51, 60, 65) vs INC 50 (GS 60)	1.350	0.921	3.662	0.999	No
DMSO Control (GS 39, 51, 60, 65) vs TDZ-K 25 (GS 60+65+2W)	0.132	0.090	3.662	1.000	No
DMSO Control (GS 39, 51, 60, 65) vs INC 25 (GS 51)	0.091	0.062	3.662	1.000	No
Nil vs INC 25 (GS 65)	2.933	2.000	3.662	0.724	No
Nil vs INC 25 (GS 39)	2.844	1.939	3.662	0.759	No
Nil vs INC 10 (GS 65)	2.184	1.489	3.662	0.945	No
Nil vs INC 25 (GS 39, 51, 60, 65)	1.940	1.322	3.662	0.976	No
Nil vs INC 50 (GS 60)	1.265	0.863	3.662	0.999	No
Nil vs TDZ-K 25 (GS 60+65+2W)	0.047	0.032	3.662	1.000	No
Nil vs INC 25 (GS 51)	0.006	0.004	3.662	1.000	No
Tukey's d critical value:			5.179		

Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
INC 25 (GS 60)	35.305	1.037	33.165	37.445	A
DMSO Control (GS 60, 65+2W)	35.233	1.037	33.093	37.374	A
TDZ-K 10 (GS 60, 65+2W)	34.558	1.037	32.417	36.698	A
INC 50 (GS 65)	33.828	1.037	31.688	35.969	A
DMSO Control (GS 39, 51, 60, 65)	33.061	1.037	30.921	35.201	A
Nil	32.976	1.037	30.836	35.116	A
INC 25 (GS 51)	32.970	1.037	30.830	35.110	A
TDZ-K 25 (GS 60+65+2W)	32.929	1.037	30.789	35.069	A
INC 50 (GS 60)	31.711	1.037	29.571	33.851	A
INC 25 (GS 39, 51, 60, 65)	31.037	1.037	28.896	33.177	A
INC 10 (GS 65)	30.793	1.037	28.652	32.933	A
INC 25 (GS 39)	30.133	1.037	27.992	32.273	A
INC 25 (GS 65)	30.043	1.037	27.903	32.184	A
Summary of all pairwise comparisons for Treatment (Tukey (HSD)):					
Category	LS means(Large TGW Overall (g))	Groups			
INC 25 (GS 60)	35.305	A			
DMSO Control (GS 60, 65+2W)	35.233	A			
TDZ-K 10 (GS 60, 65+2W)	34.558	A			
INC 50 (GS 65)	33.828	A			
DMSO Control (GS 39, 51, 60, 65)	33.061	A			
Nil	32.976	A			
INC 25 (GS 51)	32.970	A			
TDZ-K 25 (GS 60+65+2W)	32.929	A			
INC 50 (GS 60)	31.711	A			
INC 25 (GS 39, 51, 60, 65)	31.037	A			
INC 10 (GS 65)	30.793	A			
INC 25 (GS 39)	30.133	A			
INC 25 (GS 65)	30.043	A			

4.2.8 Trial 1 wheat: Small TGW Overall



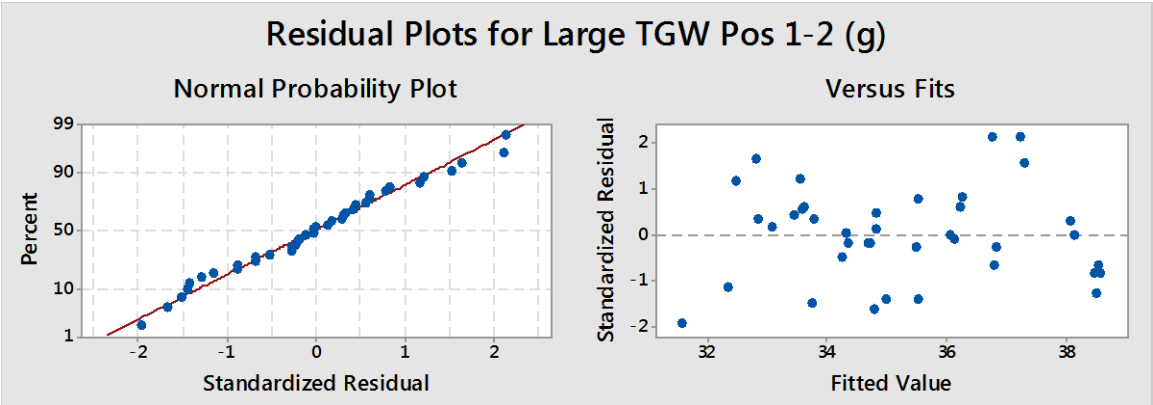
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	3.085	1.542	0.58	0.568
Treatment	12	164.053	13.671	5.14	**0.000**
Error	24	63.864	2.661		
Total	38	231.002			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Small TGW Overall (g)):								
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant			
INC 50 (GS 60) vs DMSO Control (GS 60, 65+2W)	4.876	3.661	3.662	0.050	No			
INC 50 (GS 60) vs DMSO Control (GS 39, 51, 60, 65)	3.988	2.994	3.662	0.189	No			
INC 50 (GS 60) vs Nil	2.004	1.505	3.662	0.941	No			
INC 25 (GS 60) vs DMSO Control (GS 60, 65+2W)	4.051	3.042	3.662	0.173	No			
INC 25 (GS 60) vs DMSO Control (GS 39, 51, 60, 65)	3.163	2.375	3.662	0.491	No			
INC 25 (GS 60) vs Nil	1.180	0.886	3.662	0.999	No			
INC 25 (GS 65) vs DMSO Control (GS 60, 65+2W)	2.929	2.199	3.662	0.601	No			
INC 25 (GS 65) vs DMSO Control (GS 39, 51, 60, 65)	2.041	1.532	3.662	0.934	No			
INC 25 (GS 65) vs Nil	0.058	0.043	3.662	1.000	No			
Nil vs TDZ-K 10 (GS 60, 65+2W)	5.223	3.922	3.662	**0.028**	Yes			
Nil vs INC 25 (GS 39, 51, 60, 65)	4.029	3.025	3.662	0.179	No			
Nil vs INC 10 (GS 65)	3.418	2.566	3.662	0.379	No			
Nil vs INC 25 (GS 39)	3.179	2.387	3.662	0.484	No			
Nil vs TDZ-K 25 (GS 60+65+2W)	1.999	1.501	3.662	0.942	No			
Nil vs INC 50 (GS 65)	1.810	1.359	3.662	0.971	No			
Nil vs INC 25 (GS 51)	0.221	0.166	3.662	1.000	No			
INC 25 (GS 51) vs DMSO Control (GS 60, 65+2W)	2.650	1.990	3.662	0.730	No			
INC 25 (GS 51) vs DMSO Control (GS 39, 51, 60, 65)	1.762	1.323	3.662	0.976	No			
INC 50 (GS 65) vs DMSO Control (GS 60, 65+2W)	1.061	0.797	3.662	1.000	No			
INC 50 (GS 65) vs DMSO Control (GS 39, 51, 60, 65)	0.173	0.130	3.662	1.000	No			
DMSO Control (GS 39, 51, 60, 65) vs TDZ-K 10 (GS 60, 65+2W)	3.240	2.433	3.662	0.456	No			
DMSO Control (GS 39, 51, 60, 65) vs INC 25 (GS 39, 51, 60, 65)	2.046	1.536	3.662	0.933	No			
DMSO Control (GS 39, 51, 60, 65) vs INC 10 (GS 65)	1.434	1.077	3.662	0.996	No			
DMSO Control (GS 39, 51, 60, 65) vs INC 25 (GS 39)	1.196	0.898	3.662	0.999	No			
DMSO Control (GS 39, 51, 60, 65) vs TDZ-K 25 (GS 60+65+2W)	0.016	0.012	3.662	1.000	No			
TDZ-K 25 (GS 60+65+2W) vs DMSO Control (GS 60, 65+2W)	0.872	0.655	3.662	1.000	No			
DMSO Control (GS 60, 65+2W) vs TDZ-K 10 (GS 60, 65+2W)	2.352	1.766	3.662	0.848	No			
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 39, 51, 60, 65)	1.158	0.869	3.662	0.999	No			
DMSO Control (GS 60, 65+2W) vs INC 10 (GS 65)	0.547	0.410	3.662	1.000	No			
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 39)	0.308	0.231	3.662	1.000	No			
Tukey's d critical value:			5.179					
Category	LS means	Standard error	Lower bound	Upper bound	Groups			

			(95%)	(95%)				
INC 50 (GS 60)	37.381	0.942	35.437	39.325	A			
INC 25 (GS 60)	36.557	0.942	34.613	38.500	A	B		
INC 25 (GS 65)	35.434	0.942	33.491	37.378	A	B	C	
Nil	35.377	0.942	33.433	37.320	A	B	C	
INC 25 (GS 51)	35.156	0.942	33.212	37.099	A	B	C	
INC 50 (GS 65)	33.567	0.942	31.623	35.510	A	B	C	D
DMSO Control (GS 39, 51, 60, 65)	33.393	0.942	31.450	35.337	A	B	C	D
TDZ-K 25 (GS 60+65+2W)	33.378	0.942	31.434	35.322	A	B	C	D
DMSO Control (GS 60, 65+2W)	32.506	0.942	30.562	34.449	A	B	C	D
INC 25 (GS 39)	32.198	0.942	30.254	34.142		B	C	D
INC 10 (GS 65)	31.959	0.942	30.015	33.903		B	C	D
INC 25 (GS 39, 51, 60, 65)	31.348	0.942	29.404	33.292			C	D
TDZ-K 10 (GS 60, 65+2W)	30.153	0.942	28.210	32.097				D
Summary of all pairwise comparisons for Treatment (Tukey (HSD)):								
Category	LS means(Small TGW Overall (g))	Groups						
INC 50 (GS 60)	37.381	A						
INC 25 (GS 60)	36.557	A	B					
INC 25 (GS 65)	35.434	A	B	C				
Nil	35.377	A	B	C				
INC 25 (GS 51)	35.156	A	B	C				
INC 50 (GS 65)	33.567	A	B	C	D			
DMSO Control (GS 39, 51, 60, 65)	33.393	A	B	C	D			
TDZ-K 25 (GS 60+65+2W)	33.378	A	B	C	D			
DMSO Control (GS 60, 65+2W)	32.506	A	B	C	D			
INC 25 (GS 39)	32.198		B	C	D			
INC 10 (GS 65)	31.959		B	C	D			
INC 25 (GS 39, 51, 60, 65)	31.348			C	D			
TDZ-K 10 (GS 60, 65+2W)	30.153				D			

4.2.9 Trial 1 wheat: Large TGW Pos 1-2



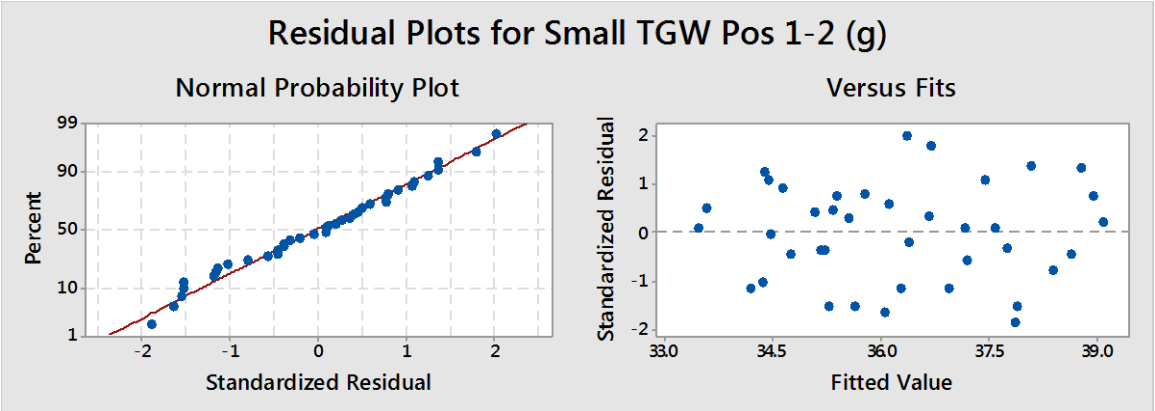
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	13.93	6.966	2.06	0.149
Treatment	12	131.48	10.956	3.25	0.007
Error	24	81.02	3.376		
Total	38	226.42			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Large TGW Pos 1-2 (g)):						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 65)	5.762	3.841	3.662	**0.034**	Yes	
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 39, 51, 60, 65)	4.981	3.320	3.662	0.102	No	
DMSO Control (GS 60, 65+2W) vs INC 10 (GS 65)	4.826	3.217	3.662	0.125	No	
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 39)	4.247	2.831	3.662	0.251	No	
DMSO Control (GS 60, 65+2W) vs INC 50 (GS 60)	3.879	2.586	3.662	0.369	No	
DMSO Control (GS 60, 65+2W) vs TDZ-K 25 (GS 60+65+2W)	3.780	2.520	3.662	0.405	No	
DMSO Control (GS 60, 65+2W) vs INC 50 (GS 65)	2.322	1.548	3.662	0.929	No	
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 51)	1.792	1.195	3.662	0.989	No	
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 60)	0.464	0.310	3.662	1.000	No	
DMSO Control (GS 60, 65+2W) vs TDZ-K 10 (GS 60, 65+2W)	0.082	0.055	3.662	1.000	No	
TDZ-K 10 (GS 60, 65+2W) vs Nil	2.983	1.988	3.662	0.730	No	
TDZ-K 10 (GS 60, 65+2W) vs DMSO Control (GS 39, 51, 60, 65)	2.396	1.597	3.662	0.914	No	
INC 25 (GS 60) vs Nil	2.601	1.734	3.662	0.862	No	
INC 25 (GS 60) vs DMSO Control (GS 39, 51, 60, 65)	2.013	1.342	3.662	0.974	No	
INC 25 (GS 51) vs Nil	1.273	0.849	3.662	1.000	No	
INC 25 (GS 51) vs DMSO Control (GS 39, 51, 60, 65)	0.686	0.457	3.662	1.000	No	
INC 50 (GS 65) vs Nil	0.743	0.495	3.662	1.000	No	
INC 50 (GS 65) vs DMSO Control (GS 39, 51, 60, 65)	0.156	0.104	3.662	1.000	No	
DMSO Control (GS 39, 51, 60, 65) vs INC 25 (GS 65)	3.284	2.189	3.662	0.607	No	
DMSO Control (GS 39, 51, 60, 65) vs INC 25 (GS 39, 51, 60, 65)	2.503	1.669	3.662	0.889	No	
DMSO Control (GS 39, 51, 60, 65) vs INC 10 (GS 65)	2.348	1.565	3.662	0.924	No	
DMSO Control (GS 39, 51, 60, 65) vs INC 25 (GS 39)	1.769	1.179	3.662	0.990	No	
DMSO Control (GS 39, 51, 60, 65) vs INC 50 (GS 60)	1.401	0.934	3.662	0.999	No	
DMSO Control (GS 39, 51, 60, 65) vs TDZ-K 25 (GS 60+65+2W)	1.302	0.868	3.662	0.999	No	
Nil vs INC 25 (GS 65)	2.697	1.798	3.662	0.833	No	
Nil vs INC 25 (GS 39, 51, 60, 65)	1.916	1.277	3.662	0.982	No	
Nil vs INC 10 (GS 65)	1.760	1.174	3.662	0.991	No	
Nil vs INC 25 (GS 39)	1.182	0.788	3.662	1.000	No	
Nil vs INC 50 (GS 60)	0.814	0.542	3.662	1.000	No	
Nil vs TDZ-K 25 (GS 60+65+2W)	0.715	0.477	3.662	1.000	No	
Tukey's d critical value:			5.179			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
DMSO Control (GS 60, 65+2W)	38.163	1.061	35.974	40.353	A	
TDZ-K 10 (GS 60, 65+2W)	38.081	1.061	35.892	40.270	A	
INC 25 (GS 60)	37.699	1.061	35.510	39.888	A	B
INC 25 (GS 51)	36.371	1.061	34.182	38.560	A	B
INC 50 (GS 65)	35.841	1.061	33.652	38.030	A	B
DMSO Control (GS 39, 51, 60, 65)	35.686	1.061	33.496	37.875	A	B

Nil	35.098	1.061	32.909	37.288	A	B
TDZ-K 25 (GS 60+65+2W)	34.383	1.061	32.194	36.573	A	B
INC 50 (GS 60)	34.284	1.061	32.095	36.474	A	B
INC 25 (GS 39)	33.917	1.061	31.727	36.106	A	B
INC 10 (GS 65)	33.338	1.061	31.148	35.527	A	B
INC 25 (GS 39, 51, 60, 65)	33.182	1.061	30.993	35.372	A	B
INC 25 (GS 65)	32.401	1.061	30.212	34.590		B

4.2.10 Trial 1 wheat: Small TGW Pos 1-2



Analysis of Variance

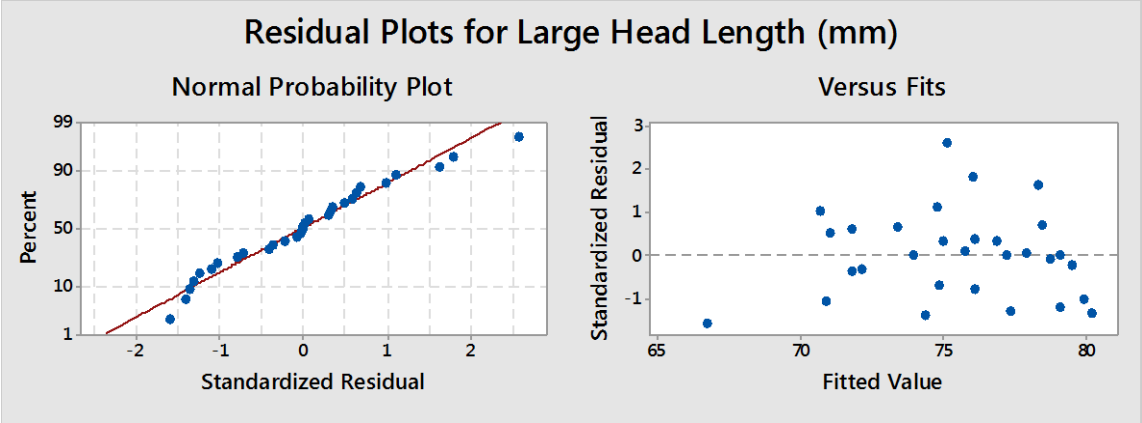
Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	9.914	4.957	2.06	0.149
Treatment	12	83.836	6.986	2.90	**0.013**
Error	24	57.765	2.407		
Total	38	151.515			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Small TGW Pos 1-2 (g)):					
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
INC 25 (GS 60) vs DMSO Control (GS 39, 51, 60, 65)	3.524	2.782	3.662	0.272	No
INC 25 (GS 60) vs Nil	2.733	2.158	3.662	0.627	No
INC 25 (GS 60) vs DMSO Control (GS 60, 65+2W)	0.696	0.549	3.662	1.000	No
INC 25 (GS 65) vs DMSO Control (GS 39, 51, 60, 65)	3.386	2.673	3.662	0.324	No
INC 25 (GS 65) vs Nil	2.594	2.048	3.662	0.695	No
INC 25 (GS 65) vs DMSO Control (GS 60, 65+2W)	0.557	0.439	3.662	1.000	No
DMSO Control (GS 60, 65+2W) vs TDZ-K 10 (GS 60, 65+2W)	3.744	2.956	3.662	0.202	No
DMSO Control (GS 60, 65+2W) vs INC 10 (GS 65)	3.631	2.867	3.662	0.236	No
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 39, 51, 60, 65)	3.002	2.370	3.662	0.494	No
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 39)	2.752	2.173	3.662	0.617	No
DMSO Control (GS 60, 65+2W) vs INC 50 (GS 65)	1.996	1.575	3.662	0.921	No
DMSO Control (GS 60, 65+2W) vs INC 25 (GS 51)	1.439	1.136	3.662	0.993	No
DMSO Control (GS 60, 65+2W) vs TDZ-K 25 (GS 60+65+2W)	0.930	0.734	3.662	1.000	No
DMSO Control (GS 60, 65+2W) vs INC 50 (GS 60)	0.520	0.411	3.662	1.000	No
INC 50 (GS 60) vs DMSO Control (GS 39, 51, 60, 65)	2.309	1.823	3.662	0.820	No
INC 50 (GS 60) vs Nil	1.518	1.198	3.662	0.989	No
TDZ-K 25 (GS 60+65+2W) vs DMSO Control (GS 39, 51, 60, 65)	1.899	1.499	3.662	0.943	No
TDZ-K 25 (GS 60+65+2W) vs Nil	1.108	0.875	3.662	0.999	No
INC 25 (GS 51) vs DMSO Control (GS 39, 51, 60, 65)	1.390	1.097	3.662	0.995	No
INC 25 (GS 51) vs Nil	0.599	0.473	3.662	1.000	No
INC 50 (GS 65) vs DMSO Control (GS 39, 51, 60, 65)	0.833	0.658	3.662	1.000	No
INC 50 (GS 65) vs Nil	0.042	0.033	3.662	1.000	No
Nil vs TDZ-K 10 (GS 60, 65+2W)	1.707	1.347	3.662	0.973	No
Nil vs INC 10 (GS 65)	1.593	1.258	3.662	0.984	No
Nil vs INC 25 (GS 39, 51, 60, 65)	0.964	0.761	3.662	1.000	No
Nil vs INC 25 (GS 39)	0.714	0.564	3.662	1.000	No
INC 25 (GS 39) vs DMSO Control (GS 39, 51, 60, 65)	0.077	0.061	3.662	1.000	No
DMSO Control (GS 39, 51, 60, 65) vs TDZ-K 10 (GS 60, 65+2W)	0.916	0.723	3.662	1.000	No
DMSO Control (GS 39, 51, 60, 65) vs INC 10 (GS 65)	0.802	0.633	3.662	1.000	No
DMSO Control (GS 39, 51, 60, 65) vs INC 25 (GS 39, 51, 60, 65)	0.173	0.137	3.662	1.000	No
Tukey's d critical value:			5.179		
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
INC 25 (GS 60)	38.597	0.896	36.748	40.445	A

INC 25 (GS 65)	38.458	0.896	36.609	40.306	A
DMSO Control (GS 60, 65+2W)	37.901	0.896	36.052	39.750	A
INC 50 (GS 60)	37.381	0.896	35.532	39.230	A
TDZ-K 25 (GS 60+65+2W)	36.971	0.896	35.122	38.820	A
INC 25 (GS 51)	36.462	0.896	34.614	38.311	A
INC 50 (GS 65)	35.906	0.896	34.057	37.754	A
Nil	35.863	0.896	34.015	37.712	A
INC 25 (GS 39)	35.149	0.896	33.300	36.998	A
DMSO Control (GS 39, 51, 60, 65)	35.072	0.896	33.224	36.921	A
INC 25 (GS 39, 51, 60, 65)	34.899	0.896	33.050	36.748	A
INC 10 (GS 65)	34.270	0.896	32.421	36.119	A
TDZ-K 10 (GS 60, 65+2W)	34.157	0.896	32.308	36.005	A

4.3 Trial 2A wheat

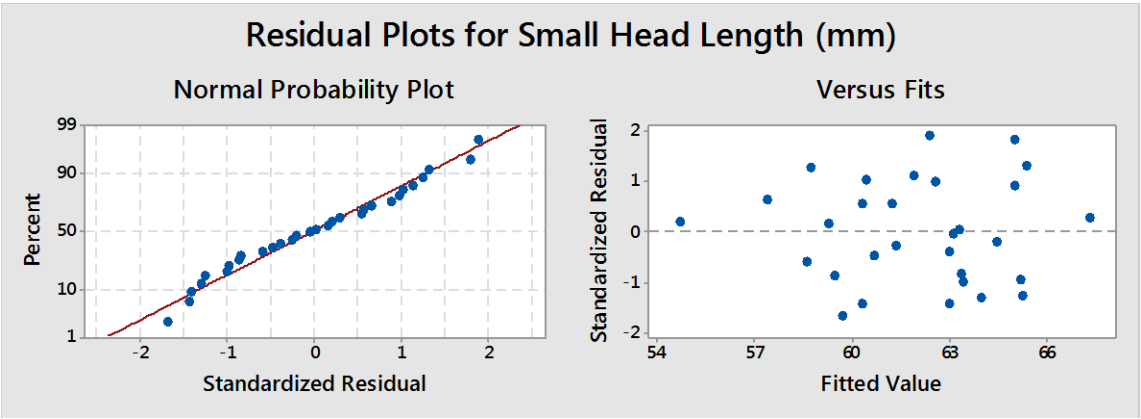
4.3.1 Trial 2A wheat: Large Head Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	142.1	71.04	3.89	0.039
Treatment	9	172.3	19.14	1.05	0.442
Error	18	328.6	18.26		
Total	29	643.0			

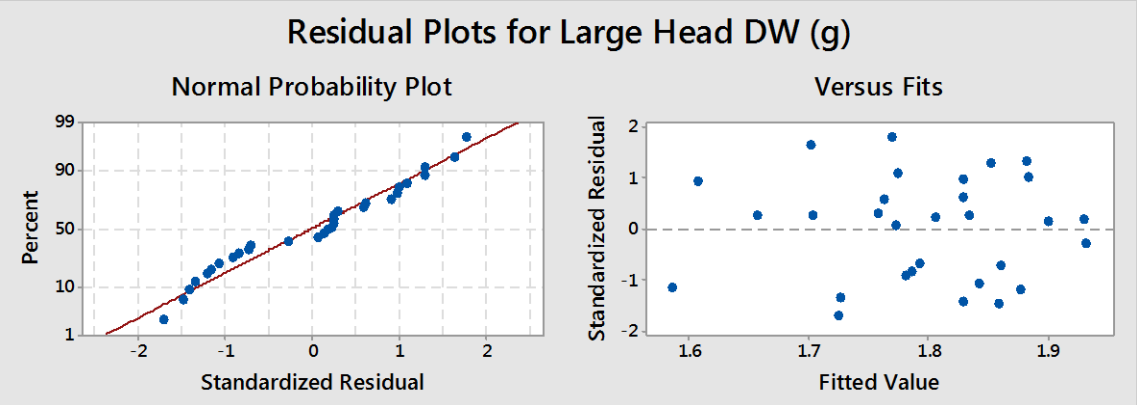
4.3.2 Trial 2A wheat: Small Head Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	111.3	55.64	3.56	0.050
Treatment	9	112.7	12.53	0.80	0.620
Error	18	281.0	15.61		
Total	29	505.0			

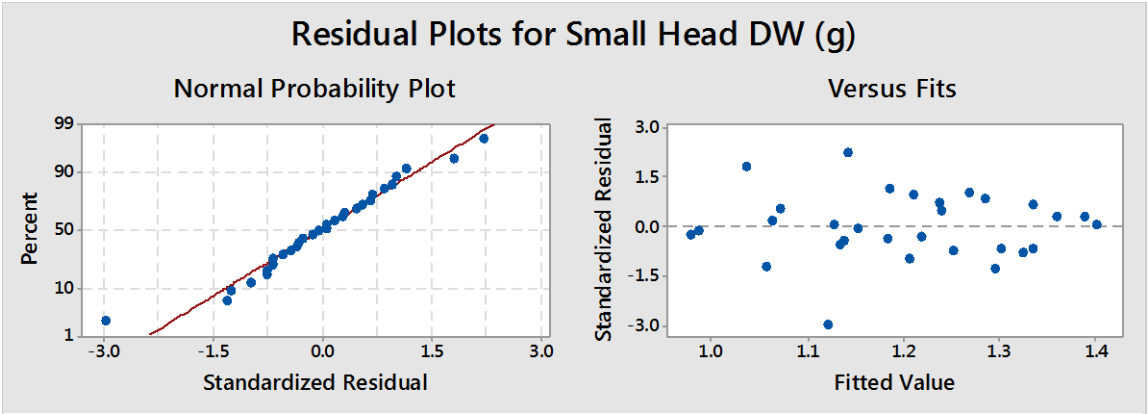
4.3.3 Trial 2A wheat: Large Head DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.02649	0.01325	0.62	0.551
Treatment	9	0.19244	0.02138	0.99	0.479
Error	18	0.38750	0.02153		
Total	29	0.60644			

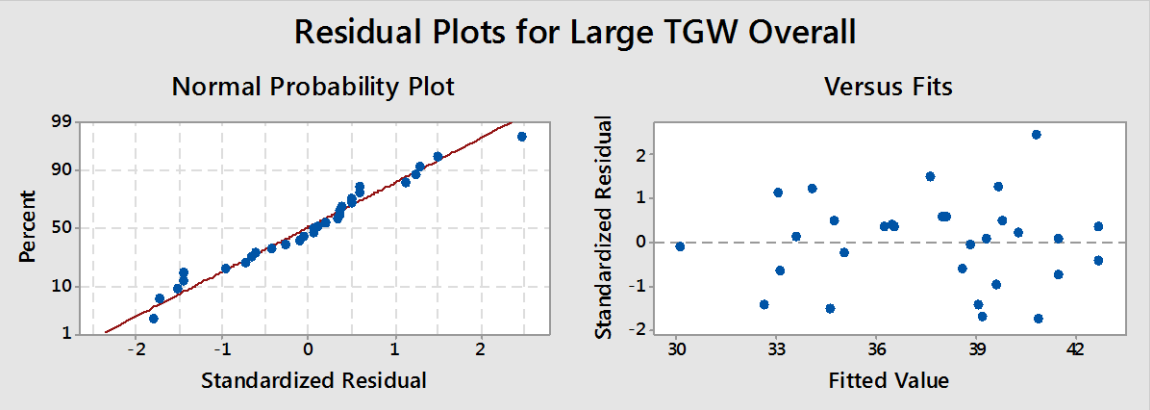
4.3.4 Trial 2A wheat: Small Head DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.1135	0.05674	3.60	0.048
Treatment	9	0.2822	0.03136	1.99	0.103
Error	18	0.2838	0.01577		
Total	29	0.6796			

4.3.5 Trial 2A wheat: Large TGW Overall

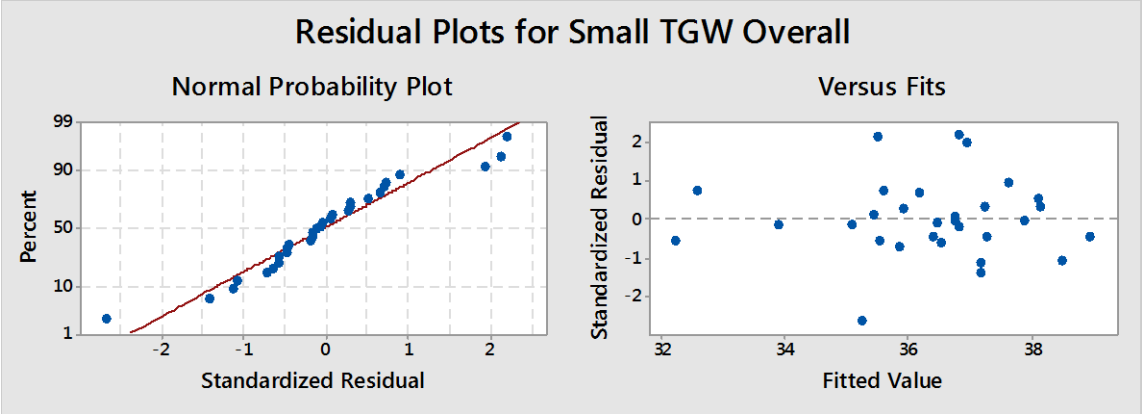


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	214.18	107.090	32.08	0.000
Treatment	9	98.04	10.893	3.26	**0.016**
Error	18	60.08	3.338		
Total	29	372.30			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
TDZ-K 10 µM (GS 61,65+2W) vs Nil	2.936	1.968	3.585	0.629	No	
CPPU 10 µM (GS 61, 65) vs Nil	2.913	1.953	3.585	0.638	No	
TDZ-K 50 µM (GS 61,65+2W) vs Nil	1.047	0.702	3.585	0.999	No	
CPPU 30 µM (GS 61, 65) vs Nil	0.495	0.332	3.585	1.000	No	
Nil vs CPPU 100 µM (GS 51, 65)	3.523	2.362	3.585	0.400	No	
Nil vs CPPU 100 µM (GS 61, 65)	0.963	0.646	3.585	1.000	No	
Nil vs CPPU 10 µM (GS 51, 65)	0.551	0.369	3.585	1.000	No	
Nil vs CPPU 30 µM (GS 51, 65)	0.467	0.313	3.585	1.000	No	
TDZ-K 10 µM (GS 61,65+2W) vs DMSO Control (GS 61, 65,+2W)	1.803	1.209	3.585	0.961	No	
CPPU 10 µM (GS 61, 65) vs DMSO Control (GS 61, 65,+2W)	1.781	1.194	3.585	0.964	No	
DMSO Control (GS 61, 65,+2W) vs CPPU 100 µM (GS 51, 65)	4.656	3.121	3.585	0.120	No	
DMSO Control (GS 61, 65,+2W) vs CPPU 100 µM (GS 61, 65)	2.096	1.405	3.585	0.910	No	
DMSO Control (GS 61, 65,+2W) vs CPPU 10 µM (GS 51, 65)	1.683	1.128	3.585	0.975	No	
DMSO Control (GS 61, 65,+2W) vs CPPU 30 µM (GS 51, 65)	1.600	1.073	3.585	0.982	No	
DMSO Control (GS 61, 65,+2W) vs CPPU 30 µM (GS 61, 65)	0.638	0.428	3.585	1.000	No	
DMSO Control (GS 61, 65,+2W) vs TDZ-K 50 µM (GS 61,65+2W)	0.086	0.057	3.585	1.000	No	
Tukey's d critical value:			5.071			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
TDZ-K 10 µM (GS 61,65+2W)	40.258	1.055	38.042	42.474	A	
CPPU 10 µM (GS 61, 65)	40.235	1.055	38.019	42.451	A	
DMSO Control (GS 61, 65,+2W)	38.454	1.055	36.238	40.671	A	B
TDZ-K 50 µM (GS 61,65+2W)	38.369	1.055	36.153	40.585	A	B
CPPU 30 µM (GS 61, 65)	37.817	1.055	35.601	40.033	A	B
Nil	37.322	1.055	35.106	39.538	A	B
CPPU 30 µM (GS 51, 65)	36.854	1.055	34.638	39.071	A	B
CPPU 10 µM (GS 51, 65)	36.771	1.055	34.555	38.987	A	B
CPPU 100 µM (GS 61, 65)	36.359	1.055	34.143	38.575	A	B
CPPU 100 µM (GS 51, 65)	33.799	1.055	31.583	36.015		B

4.3.6 Trial 2A wheat: Small TGW Overall

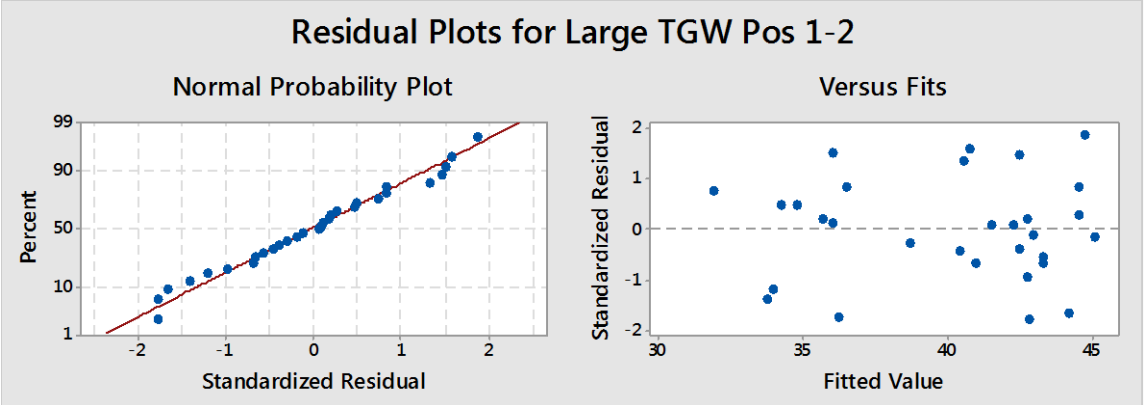


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	16.00	8.0022	9.84	0.001
Treatment	9	53.77	5.9747	7.35	**0.000**
Error	18	14.64	0.8134		
Total	29	84.42			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
CPPU 100 µM (GS 61, 65) vs Nil	1.726	2.343	3.585	0.410	No	
CPPU 10 µM (GS 51, 65) vs Nil	1.269	1.723	3.585	0.770	No	
CPPU 30 µM (GS 51, 65) vs Nil	0.861	1.169	3.585	0.968	No	
CPPU 10 µM (GS 61, 65) vs Nil	0.638	0.866	3.585	0.996	No	
Nil vs CPPU 30 µM (GS 61, 65)	**3.344**	**4.542**	**3.585**	**0.007**	**Yes**	
Nil vs TDZ-K 50 µM (GS 61,65+2W)	0.469	0.637	3.585	1.000	No	
Nil vs TDZ-K 10 µM (GS 61,65+2W)	0.302	0.410	3.585	1.000	No	
Nil vs CPPU 100 µM (GS 51, 65)	0.051	0.069	3.585	1.000	No	
CPPU 100 µM (GS 61, 65) vs DMSO Control (GS 61, 65,+2W)	0.807	1.095	3.585	0.979	No	
CPPU 10 µM (GS 51, 65) vs DMSO Control (GS 61, 65,+2W)	0.350	0.475	3.585	1.000	No	
DMSO Control (GS 61, 65,+2W) vs CPPU 30 µM (GS 61, 65)	**4.263**	**5.789**	**3.585**	**0.001**	**Yes**	
DMSO Control (GS 61, 65,+2W) vs TDZ-K 50 µM (GS 61,65+2W)	1.388	1.885	3.585	0.679	No	
DMSO Control (GS 61, 65,+2W) vs TDZ-K 10 µM (GS 61,65+2W)	1.221	1.658	3.585	0.804	No	
DMSO Control (GS 61, 65,+2W) vs CPPU 100 µM (GS 51, 65)	0.970	1.317	3.585	0.937	No	
DMSO Control (GS 61, 65,+2W) vs CPPU 10 µM (GS 61, 65)	0.281	0.382	3.585	1.000	No	
DMSO Control (GS 61, 65,+2W) vs CPPU 30 µM (GS 51, 65)	0.058	0.078	3.585	1.000	No	
Tukey's d critical value:			5.071			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
CPPU 100 µM (GS 61, 65)	37.963	0.521	36.869	39.057	A	
CPPU 10 µM (GS 51, 65)	37.507	0.521	36.413	38.601	A	
DMSO Control (GS 61, 65,+2W)	37.157	0.521	36.063	38.251	A	
CPPU 30 µM (GS 51, 65)	37.099	0.521	36.005	38.193	A	
CPPU 10 µM (GS 61, 65)	36.876	0.521	35.782	37.970	A	
Nil	36.238	0.521	35.144	37.332	A	
CPPU 100 µM (GS 51, 65)	36.187	0.521	35.093	37.281	A	
TDZ-K 10 µM (GS 61,65+2W)	35.936	0.521	34.842	37.030	A	
TDZ-K 50 µM (GS 61,65+2W)	35.769	0.521	34.675	36.863	A	
CPPU 30 µM (GS 61, 65)	32.893	0.521	31.799	33.987		B

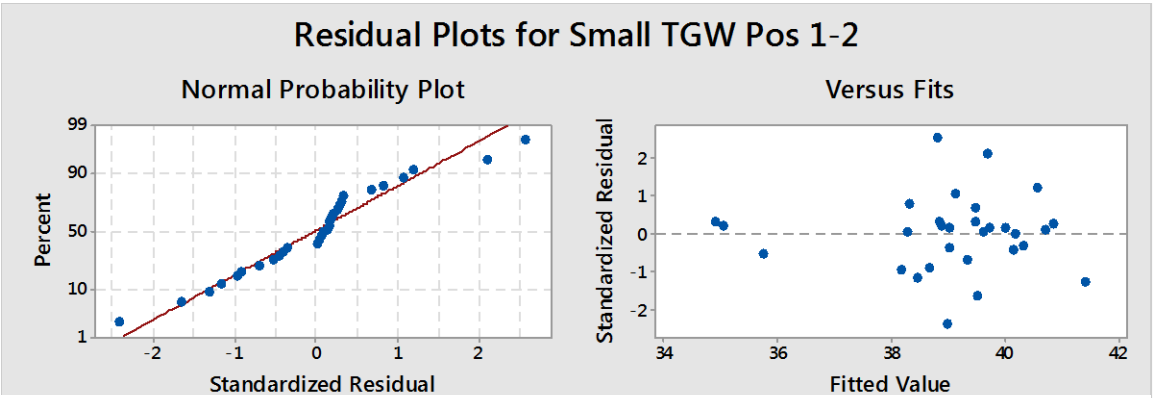
4.3.7 Trial 2A wheat: Large TGW Pos 1-2



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	404.79	202.396	73.40	0.000
Treatment	9	56.95	6.328	2.30	0.064
Error	18	49.63	2.757		
Total	29	511.38			

4.3.8 Trial 2A wheat: Small TGW Pos 1-2



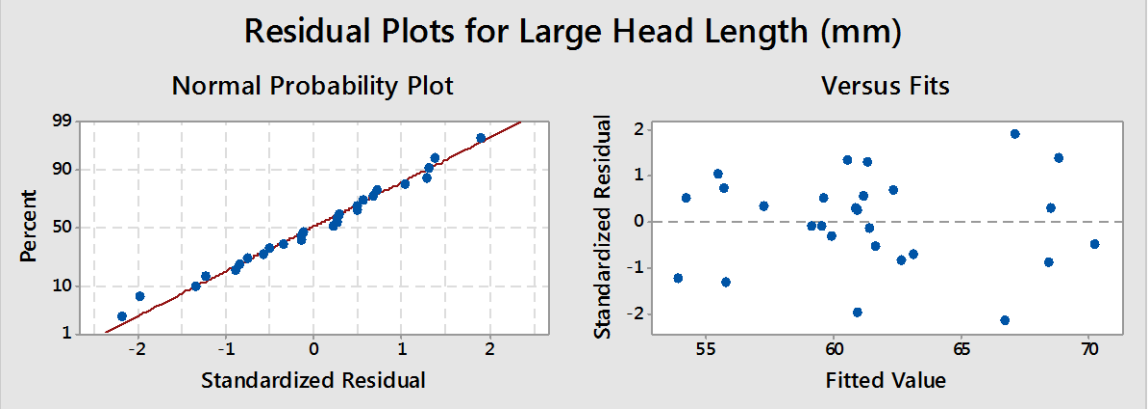
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	4.064	2.0322	2.18	0.142
Treatment	9	63.345	7.0384	7.56	**0.000**
Error	18	16.765	0.9314		
Total	29	84.175			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
CPPU 100 µM (GS 61, 65) vs Nil	2.267	2.877	3.585	0.183	No	
CPPU 30 µM (GS 51, 65) vs Nil	1.697	2.153	3.585	0.517	No	
CPPU 10 µM (GS 61, 65) vs Nil	1.191	1.512	3.585	0.871	No	
CPPU 10 µM (GS 51, 65) vs Nil	1.046	1.327	3.585	0.934	No	
TDZ-K 10 µM (GS 61,65+2W) vs Nil	0.541	0.687	3.585	0.999	No	
CPPU 100 µM (GS 51, 65) vs Nil	0.376	0.477	3.585	1.000	No	
Nil vs CPPU 30 µM (GS 61, 65)	3.399	4.313	3.585	0.012	Yes	
Nil vs TDZ-K 50 µM (GS 61,65+2W)	0.112	0.142	3.585	1.000	No	
CPPU 100 µM (GS 61, 65) vs DMSO Control (GS 61, 65,+2W)	1.677	2.128	3.585	0.532	No	
CPPU 30 µM (GS 51, 65) vs DMSO Control (GS 61, 65,+2W)	1.107	1.404	3.585	0.911	No	
CPPU 10 µM (GS 61, 65) vs DMSO Control (GS 61, 65,+2W)	0.601	0.763	3.585	0.998	No	
CPPU 10 µM (GS 51, 65) vs DMSO Control (GS 61, 65,+2W)	0.456	0.578	3.585	1.000	No	
DMSO Control (GS 61, 65,+2W) vs CPPU 30 µM (GS 61, 65)	**3.989**	**5.062**	**3.585**	**0.002**	**Yes**	
DMSO Control (GS 61, 65,+2W) vs TDZ-K 50 µM (GS 61,65+2W)	0.702	0.891	3.585	0.995	No	
DMSO Control (GS 61, 65,+2W) vs CPPU 100 µM (GS 51, 65)	0.214	0.272	3.585	1.000	No	
DMSO Control (GS 61, 65,+2W) vs TDZ-K 10 µM (GS 61,65+2W)	0.049	0.062	3.585	1.000	No	
Tukey's d critical value:			5.071			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
CPPU 100 µM (GS 61, 65)	40.909	0.557	39.738	42.080	A	
CPPU 30 µM (GS 51, 65)	40.339	0.557	39.168	41.510	A	
CPPU 10 µM (GS 61, 65)	39.833	0.557	38.663	41.004	A	
CPPU 10 µM (GS 51, 65)	39.688	0.557	38.517	40.858	A	
DMSO Control (GS 61, 65,+2W)	39.232	0.557	38.062	40.403	A	
TDZ-K 10 µM (GS 61,65+2W)	39.183	0.557	38.013	40.354	A	
CPPU 100 µM (GS 51, 65)	39.018	0.557	37.847	40.188	A	
Nil	38.642	0.557	37.472	39.813	A	
TDZ-K 50 µM (GS 61,65+2W)	38.530	0.557	37.359	39.701	A	
CPPU 30 µM (GS 61, 65)	35.243	0.557	34.073	36.414		B

4.4 Trial 2B barley

4.4.1 Trial 2B barley: Large Head Length

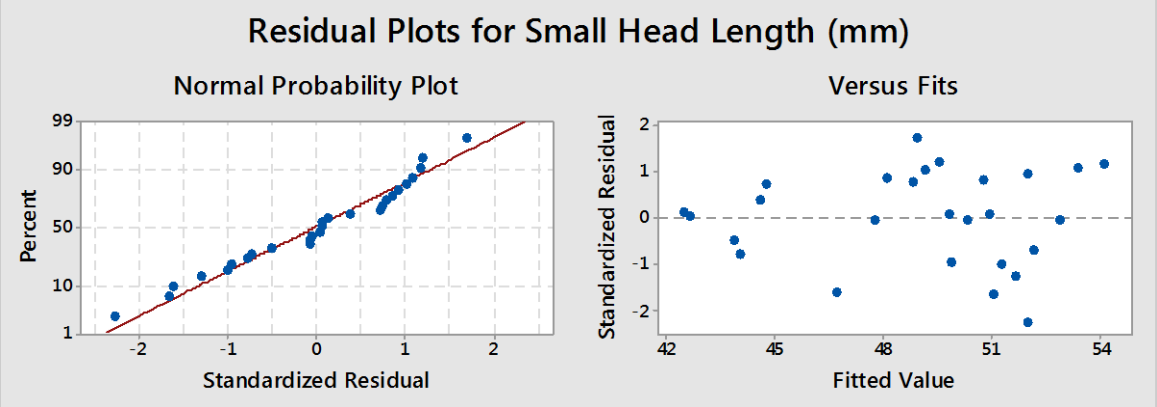


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	16.56	8.281	0.71	0.509
Treatment	8	530.92	66.365	5.66	**0.002**
Error	16	187.76	11.735		
Total	26	735.25			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
Nil vs INCYDE 25 µM (GS 65)	7.008	2.506	3.558	0.298	No	
Nil vs INCYDE 25 µM (GS 61)	5.441	1.945	3.558	0.596	No	
Nil vs INCYDE 25 µM (GS 51)	1.690	0.604	3.558	0.999	No	
Nil vs INCYDE 10 µM (GS 65)	1.270	0.454	3.558	1.000	No	
Nil vs INCYDE 50 µM (GS 65)	0.286	0.102	3.558	1.000	No	
INCYDE 25 µM (GS 39) vs Nil	7.659	2.738	3.558	0.209	No	
INCYDE 25 µM (GS 39, 51, 61, 65) vs Nil	5.929	2.120	3.558	0.494	No	
INCYDE 25 µM (GS 39) vs DMSO Control (GS 39, 51, 61, 65)	7.206	2.576	3.558	0.268	No	
INCYDE 25 µM (GS 39, 51, 61, 65) vs DMSO Control (GS 39, 51, 61, 65)	5.476	1.958	3.558	0.589	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 65)	7.460	2.667	3.558	0.234	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 61)	5.894	2.107	3.558	0.501	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 51)	2.143	0.766	3.558	0.996	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 10 µM (GS 65)	1.722	0.616	3.558	0.999	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 50 µM (GS 65)	0.738	0.264	3.558	1.000	No	
Tukey's d critical value:			5.031			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
INCYDE 25 µM (GS 39)	69.214	1.978	65.022	73.407	A	
INCYDE 25 µM (GS 39, 51, 61, 65)	67.484	1.978	63.291	71.677	A	
DMSO Control (GS 39, 51, 61, 65)	62.008	1.978	57.815	66.201	A	B
Nil	61.556	1.978	57.363	65.748	A	B
INCYDE 50 µM (GS 65)	61.270	1.978	57.077	65.463	A	B
INCYDE 10 µM (GS 65)	60.286	1.978	56.093	64.478	A	B
INCYDE 25 µM (GS 51)	59.865	1.978	55.672	64.058	A	B
INCYDE 25 µM (GS 61)	56.114	1.978	51.922	60.307		B
INCYDE 25 µM (GS 65)	54.548	1.978	50.355	58.740		B

4.4.2 Trial 2B barley: Small Head Length

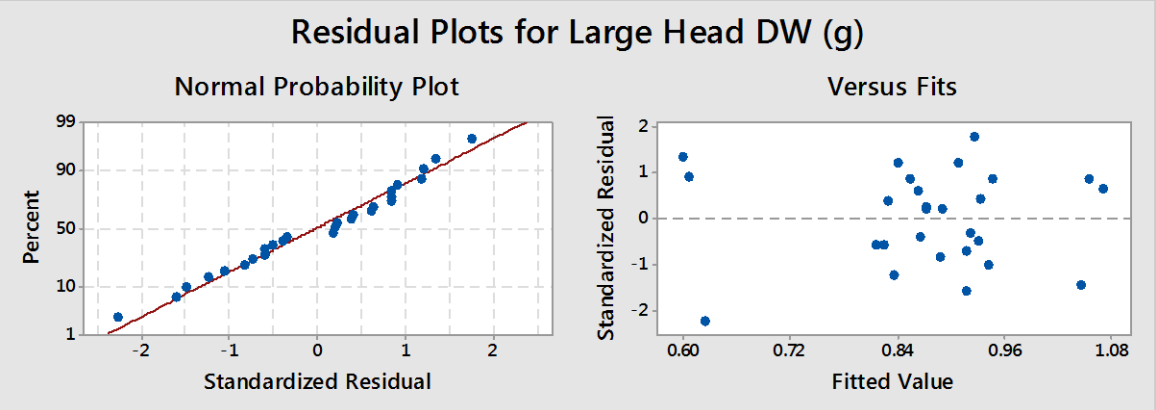


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	21.02	10.508	1.55	0.242
Treatment	8	273.75	34.218	5.05	**0.003**
Error	16	108.40	6.775		
Total	26	403.16			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
INCYDE 25 µM (GS 39, 51, 61, 65) vs DMSO Control (GS 39, 51, 61, 65)	1.250	0.588	3.558	0.999	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 61)	8.292	3.901	3.558	0.026	Yes	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 65)	8.125	3.823	3.558	0.030	Yes	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 50 µM (GS 65)	3.000	1.412	3.558	0.878	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 10 µM (GS 65)	1.833	0.863	3.558	0.992	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 51)	1.208	0.569	3.558	1.000	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 39)	0.917	0.431	3.558	1.000	No	
INCYDE 25 µM (GS 39) vs Nil	3.125	1.470	3.558	0.853	No	
INCYDE 25 µM (GS 51) vs Nil	2.833	1.333	3.558	0.907	No	
INCYDE 10 µM (GS 65) vs Nil	2.208	1.039	3.558	0.975	No	
INCYDE 50 µM (GS 65) vs Nil	1.042	0.490	3.558	1.000	No	
Nil vs INCYDE 25 µM (GS 61)	4.250	2.000	3.558	0.564	No	
Nil vs INCYDE 25 µM (GS 65)	4.083	1.921	3.558	0.610	No	
INCYDE 25 µM (GS 39, 51, 61, 65) vs Nil	5.292	2.490	3.558	0.305	No	
Tukey's d critical value:			5.031			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
INCYDE 25 µM (GS 39, 51, 61, 65)	53.208	1.503	50.023	56.394	A	
DMSO Control (GS 39, 51, 61, 65)	51.958	1.503	48.773	55.144	A	
INCYDE 25 µM (GS 39)	51.042	1.503	47.856	54.227	A	B
INCYDE 25 µM (GS 51)	50.750	1.503	47.564	53.936	A	B
INCYDE 10 µM (GS 65)	50.125	1.503	46.939	53.311	A	B
INCYDE 50 µM (GS 65)	48.958	1.503	45.773	52.144	A	B
Nil	47.917	1.503	44.731	51.102	A	B
INCYDE 25 µM (GS 65)	43.833	1.503	40.648	47.019		B
INCYDE 25 µM (GS 61)	43.667	1.503	40.481	46.852		B

4.4.3 Trial 2B barley: Large Head DW

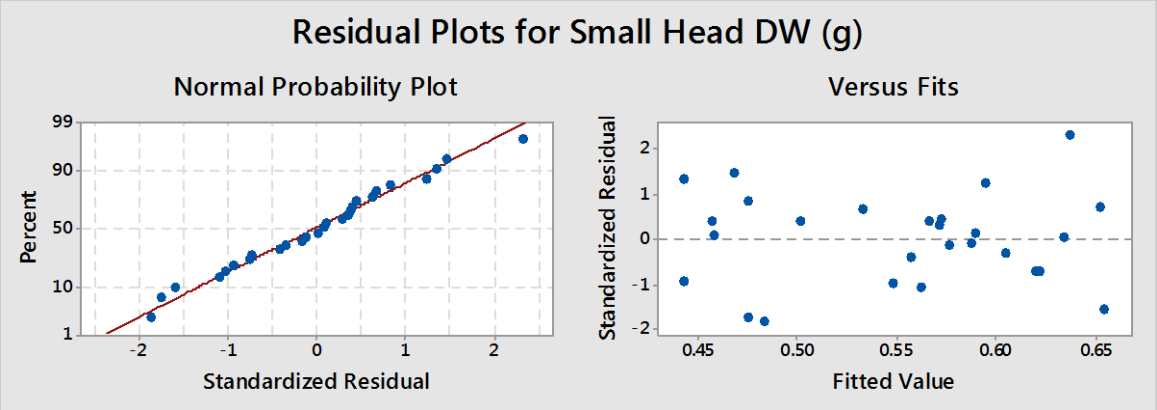


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.002862	0.001431	0.19	0.825
Treatment	8	0.347070	0.043384	5.90	**0.001**
Error	16	0.117670	0.007354		
Total	26	0.467602			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
INCYDE 25 µM (GS 39) vs Nil	0.183	2.608	3.558	0.256	No	
INCYDE 25 µM (GS 39, 51, 61, 65) vs Nil	0.058	0.834	3.558	0.994	No	
INCYDE 10 µM (GS 65) vs Nil	0.044	0.632	3.558	0.999	No	
INCYDE 25 µM (GS 51) vs Nil	0.001	0.015	3.558	1.000	No	
Nil vs INCYDE 25 µM (GS 65)	0.266	3.793	3.558	0.032	Yes	
Nil vs INCYDE 50 µM (GS 65)	0.048	0.687	3.558	0.998	No	
Nil vs INCYDE 25 µM (GS 61)	0.036	0.507	3.558	1.000	No	
INCYDE 25 µM (GS 39) vs DMSO Control (GS 39, 51, 61, 65)	0.129	1.841	3.558	0.658	No	
INCYDE 25 µM (GS 39, 51, 61, 65) vs DMSO Control (GS 39, 51, 61, 65)	0.005	0.068	3.558	1.000	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 65)	0.319	4.559	3.558	0.007	Yes	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 50 µM (GS 65)	0.102	1.453	3.558	0.861	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 61)	0.089	1.273	3.558	0.926	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 51)	0.053	0.751	3.558	0.997	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 10 µM (GS 65)	0.009	0.134	3.558	1.000	No	
Tukey's d critical value:			5.031			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
INCYDE 25 µM (GS 39)	1.058	0.050	0.953	1.163	A	
INCYDE 25 µM (GS 39, 51, 61, 65)	0.934	0.050	0.829	1.039	A	
DMSO Control (GS 39, 51, 61, 65)	0.929	0.050	0.824	1.034	A	
INCYDE 10 µM (GS 65)	0.920	0.050	0.815	1.025	A	
INCYDE 25 µM (GS 51)	0.877	0.050	0.772	0.982	A	
Nil	0.876	0.050	0.771	0.981	A	
INCYDE 25 µM (GS 61)	0.840	0.050	0.735	0.945	A	B
INCYDE 50 µM (GS 65)	0.828	0.050	0.723	0.933	A	B
INCYDE 25 µM (GS 65)	0.610	0.050	0.505	0.715		B

4.4.4 Trial 2B barley: Small Head DW

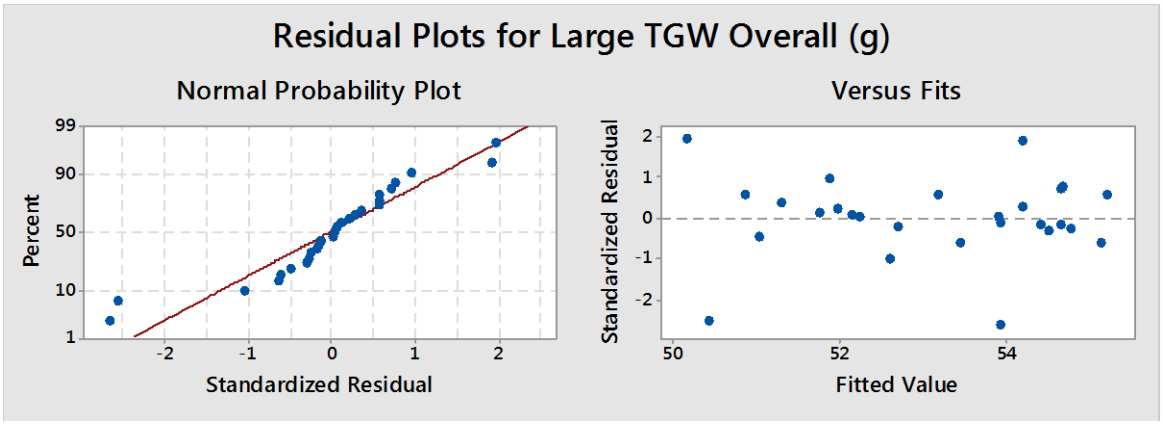


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.004786	0.002393	0.50	0.614
Treatment	8	0.116928	0.014616	3.07	**0.027**
Error	16	0.076141	0.004759		
Total	26	0.197855			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:					
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
INCYDE 25 µM (GS 39) vs Nil	0.066	1.166	3.558	0.953	No
INCYDE 25 µM (GS 51) vs Nil	0.016	0.285	3.558	1.000	No
INCYDE 10 µM (GS 65) vs Nil	0.005	0.095	3.558	1.000	No
Nil vs INCYDE 25 µM (GS 61)	0.114	2.032	3.558	0.545	No
Nil vs INCYDE 50 µM (GS 65)	0.114	2.023	3.558	0.550	No
Nil vs INCYDE 25 µM (GS 65)	0.088	1.566	3.558	0.809	No
Nil vs INCYDE 25 µM (GS 39, 51, 61, 65)	0.023	0.414	3.558	1.000	No
INCYDE 25 µM (GS 39) vs DMSO Control (GS 39, 51, 61, 65)	0.003	0.046	3.558	1.000	No
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 61)	0.177	3.151	3.558	0.105	No
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 50 µM (GS 65)	0.177	3.143	3.558	0.106	No
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 65)	0.151	2.685	3.558	0.227	No
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 39, 51, 61, 65)	0.086	1.534	3.558	0.825	No
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 10 µM (GS 65)	0.058	1.024	3.558	0.977	No
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 51)	0.047	0.834	3.558	0.994	No
Tukey's d critical value:			5.031		
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
INCYDE 25 µM (GS 39)	0.639	0.040	0.554	0.723	A
DMSO Control (GS 39, 51, 61, 65)	0.636	0.040	0.552	0.720	A
INCYDE 25 µM (GS 51)	0.589	0.040	0.505	0.673	A
INCYDE 10 µM (GS 65)	0.578	0.040	0.494	0.663	A
Nil	0.573	0.040	0.489	0.657	A
INCYDE 25 µM (GS 39, 51, 61, 65)	0.550	0.040	0.465	0.634	A
INCYDE 25 µM (GS 65)	0.485	0.040	0.400	0.569	A
INCYDE 50 µM (GS 65)	0.459	0.040	0.375	0.543	A
INCYDE 25 µM (GS 61)	0.459	0.040	0.374	0.543	A

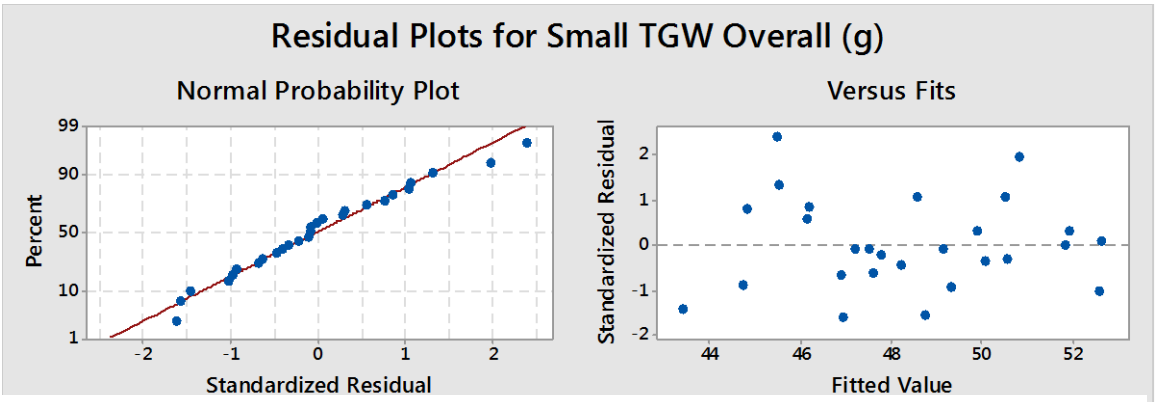
4.4.5 Trial 2B barley: Large TGW Overall



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	2.396	1.198	0.29	0.750
Treatment	8	59.632	7.454	1.82	0.147
Error	16	65.586	4.099		
Total	26	127.613			

4.4.6 Trial 2B barley: Small TGW Overall



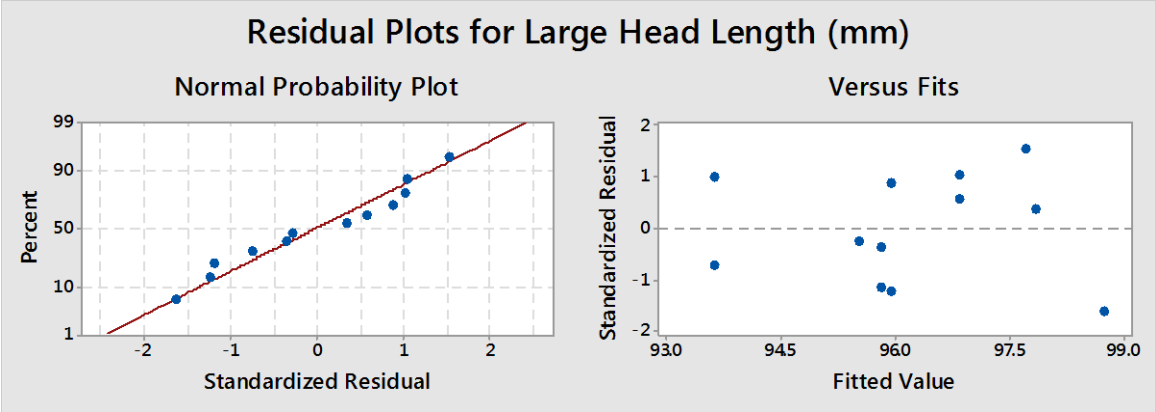
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	20.24	10.120	2.03	0.164
Treatment	8	147.28	18.410	3.70	**0.013**
Error	16	79.69	4.981		
Total	26	247.21			

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant	
Nil vs INCYDE 50 µM (GS 65)	**7.191**	**3.946**	**3.558**	**0.024**	**Yes**	
Nil vs INCYDE 25 µM (GS 65)	5.738	3.149	3.558	0.105	No	
Nil vs INCYDE 25 µM (GS 39, 51, 61, 65)	5.047	2.770	3.558	0.199	No	
Nil vs INCYDE 25 µM (GS 39)	4.428	2.430	3.558	0.332	No	
Nil vs INCYDE 25 µM (GS 61)	3.348	1.837	3.558	0.660	No	
Nil vs INCYDE 25 µM (GS 51)	2.780	1.526	3.558	0.828	No	
Nil vs INCYDE 10 µM (GS 65)	0.071	0.039	3.558	1.000	No	
INCYDE 10 µM (GS 65) vs DMSO Control (GS 39, 51, 61, 65)	1.760	0.966	3.558	0.984	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 50 µM (GS 65)	5.360	2.942	3.558	0.150	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 65)	3.907	2.144	3.558	0.480	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 39, 51, 61, 65)	3.216	1.765	3.558	0.702	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 39)	2.597	1.425	3.558	0.872	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 61)	1.517	0.832	3.558	0.994	No	
DMSO Control (GS 39, 51, 61, 65) vs INCYDE 25 µM (GS 51)	0.949	0.521	3.558	1.000	No	
Tukey's d critical value:			5.031			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
Nil	51.724	1.288	48.993	54.456	A	
INCYDE 10 µM (GS 65)	51.653	1.288	48.922	54.385	A	
DMSO Control (GS 39, 51, 61, 65)	49.893	1.288	47.162	52.625	A	B
INCYDE 25 µM (GS 51)	48.944	1.288	46.213	51.676	A	B
INCYDE 25 µM (GS 61)	48.377	1.288	45.645	51.108	A	B
INCYDE 25 µM (GS 39)	47.297	1.288	44.565	50.028	A	B
INCYDE 25 µM (GS 39, 51, 61, 65)	46.678	1.288	43.946	49.409	A	B
INCYDE 25 µM (GS 65)	45.987	1.288	43.255	48.718	A	B
INCYDE 50 µM (GS 65)	44.533	1.288	41.802	47.265		B

4.5 Drought Trial 3A wheat

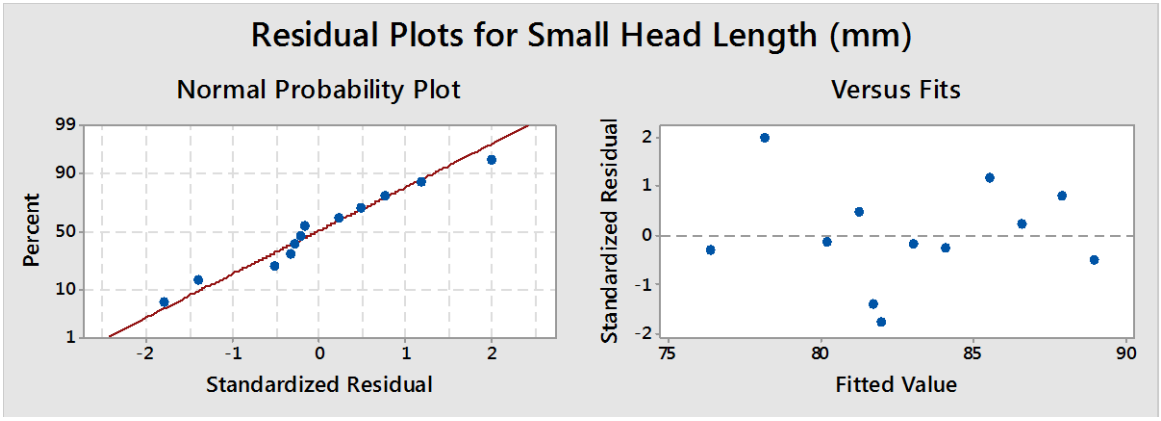
4.5.1 Drought Trial 3A wheat: Large Head Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	9.627	4.813	0.45	0.660
Treatment	3	16.976	5.659	0.52	0.682
Error	6	64.827	10.804		
Total	11	91.429			

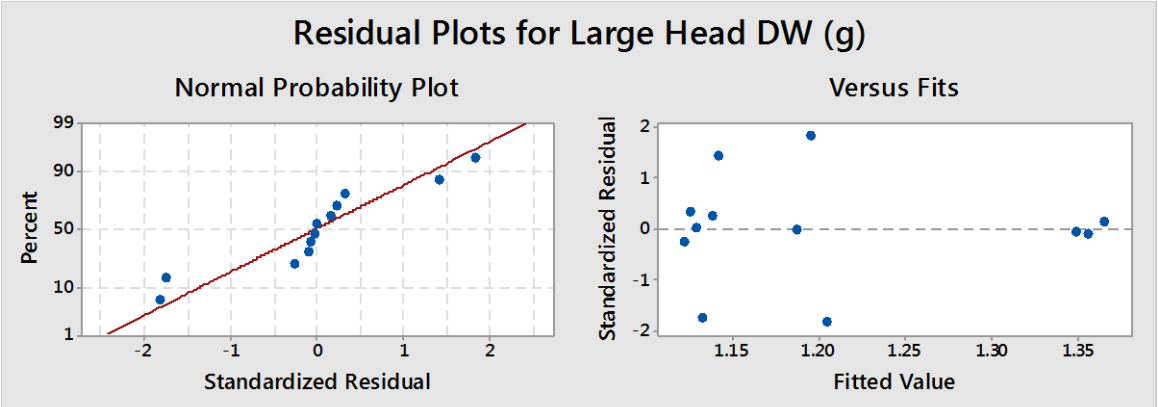
4.5.2 Drought Trial 3A wheat: Small Head Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	53.25	26.62	0.58	0.589
Treatment	3	108.70	36.23	0.79	0.543
Error	6	275.93	45.99		
Total	11	437.88			

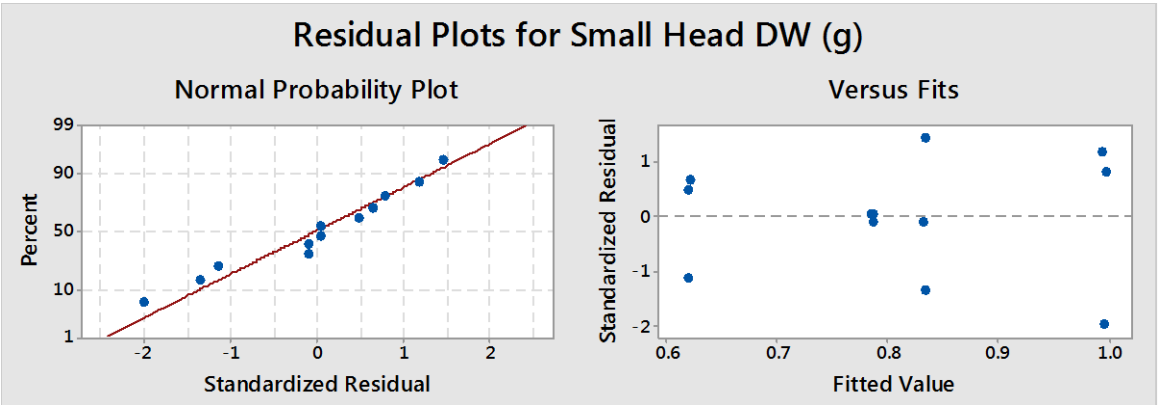
4.5.3 Drought Trial 3A wheat: Large Head DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.000558	0.000279	0.02	0.979
Treatment	3	0.102583	0.034194	2.56	0.150
Error	6	0.079990	0.013332		
Total	11	0.183131			

4.5.4 Drought Trial 3A wheat: Small Head DW



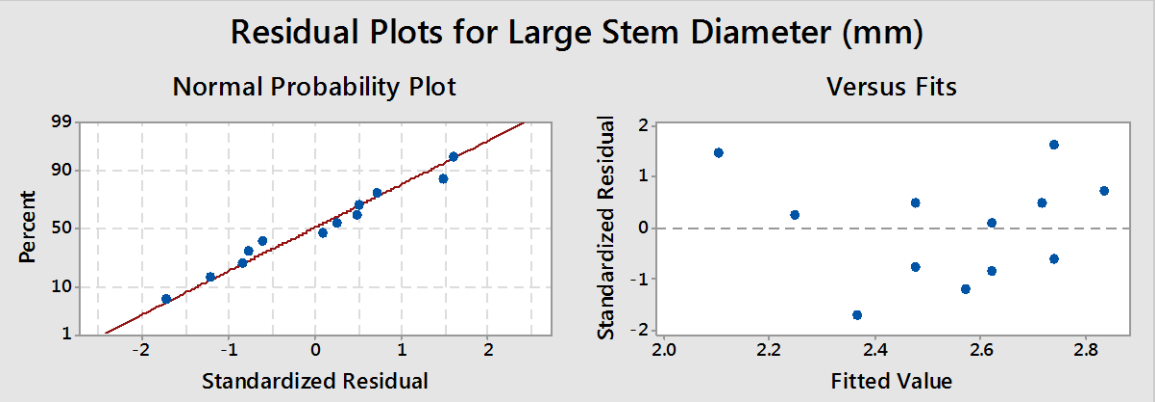
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.000008	0.000004	0.00	1.000
Treatment	3	0.215841	0.071947	7.03	**0.022**
Error	6	0.061394	0.010232		
Total	11	0.277243			

Treatment / Dunnett (two sided) / Analysis of the differences between the control category Control and the other categories with a confidence interval of 95%:

Category	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs INCYDE 50 µM	0.376	4.557	3.099	0.256	0.009	Yes
Control vs CPPU 100 µM	0.210	2.537	3.099	0.256	0.102	No
Control vs TDZ-K 50 µM	0.163	1.967	3.099	0.256	0.212	No

4.5.5 Drought Trial 3A wheat: Large Stem Diameter



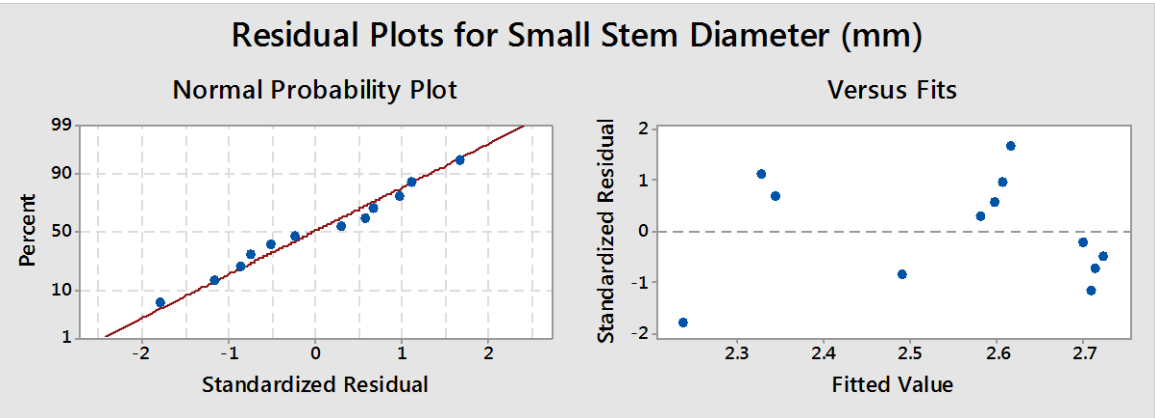
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.14087	0.07043	4.80	0.057
Treatment	3	0.38876	0.12959	8.83	**0.013**
Error	6	0.08807	0.01468		
Total	11	0.61769			

Treatment / Dunnett (two sided) / Analysis of the differences between the control category Control and the other categories with a confidence interval of 95%:

Category	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs INCYDE 50 µM	-0.097	-0.977	3.099	0.307	0.663	No
Control vs TDZ-K 50 µM	0.373	3.774	3.099	0.307	0.022	Yes
Control vs CPPU 100 µM	0.000	0.000	3.099	0.307	1.000	No

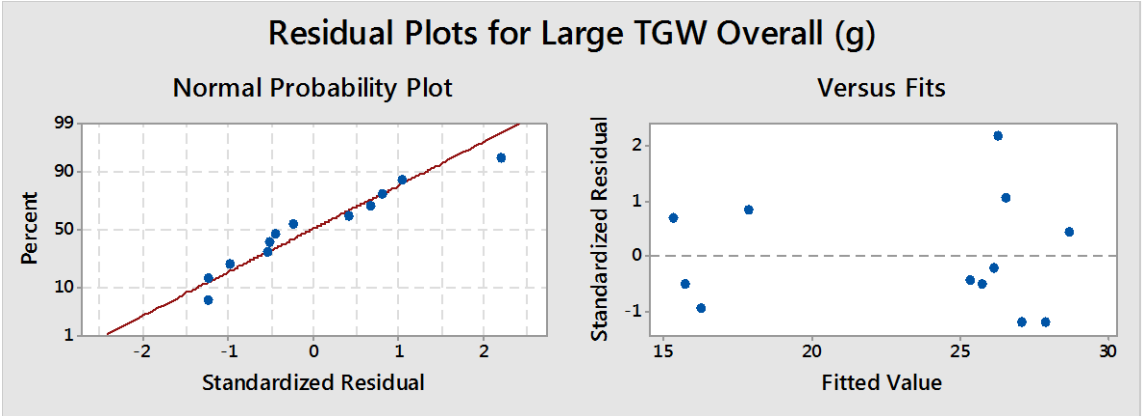
4.5.6 Drought Trial 3A wheat: Small Stem Diameter



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.02712	0.01356	0.42	0.676
Treatment	3	0.28143	0.09381	2.90	0.124
Error	6	0.19415	0.03236		
Total	11	0.50269			

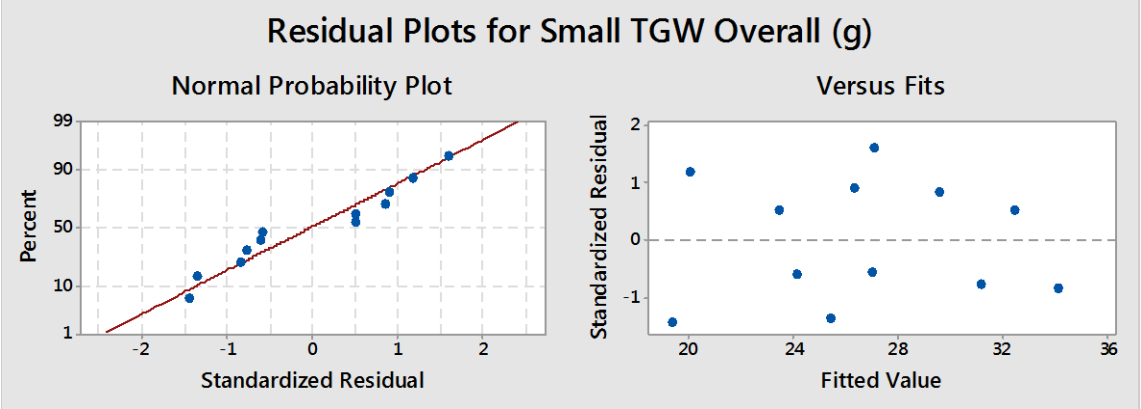
4.5.7 Drought Trial 3A wheat: Large TGW overall



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	292.632	146.316	165.42	0.000
Treatment	3	11.362	3.787	4.28	0.062
Error	6	5.307	0.885		
Total	11	309.302			

4.5.8 Drought Trial 3A wheat: Small TGW Overall

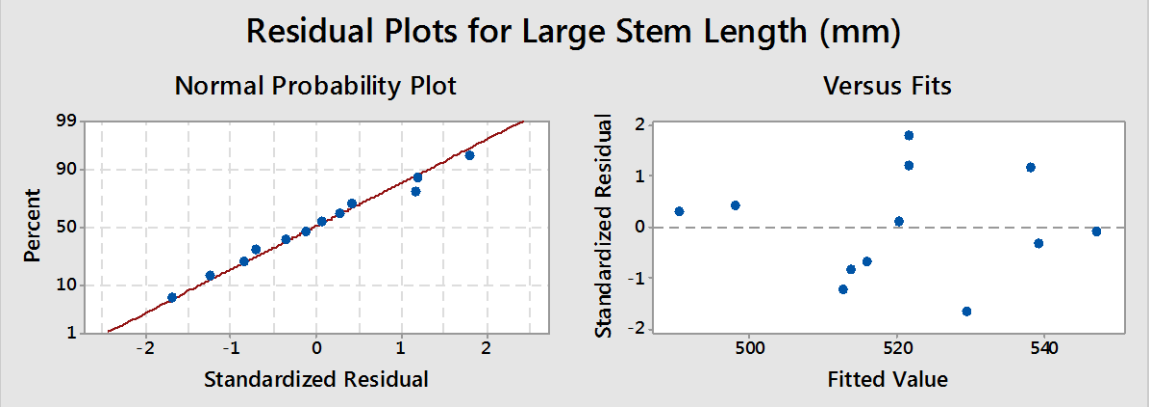


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	99.92	49.96	3.75	0.088
Treatment	3	135.71	45.24	3.40	0.094
Error	6	79.90	13.32		
Total	11	315.52			

4.6 Trial 3B barley

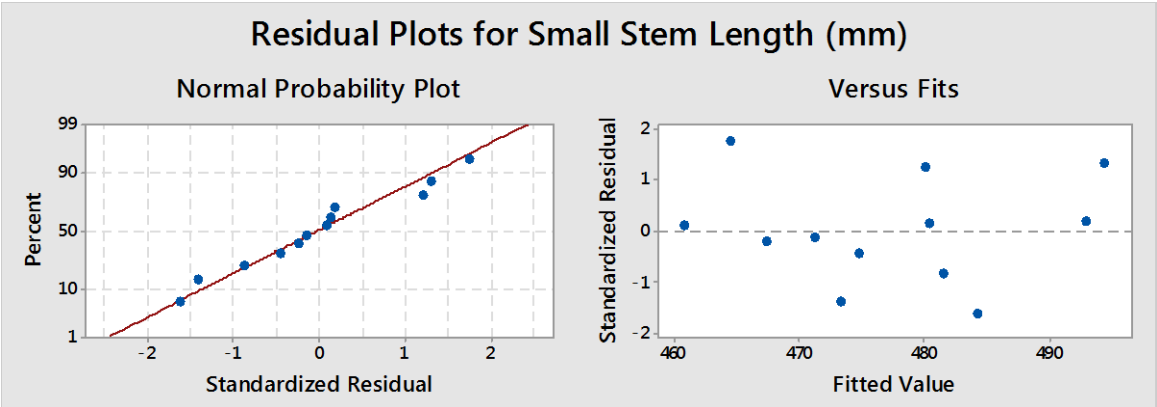
4.6.1 Drought Trial 3B barley: Large Stem Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	1368	684.0	0.88	0.462
Treatment	3	1630	543.5	0.70	0.586
Error	6	4659	776.5		
Total	11	7658			

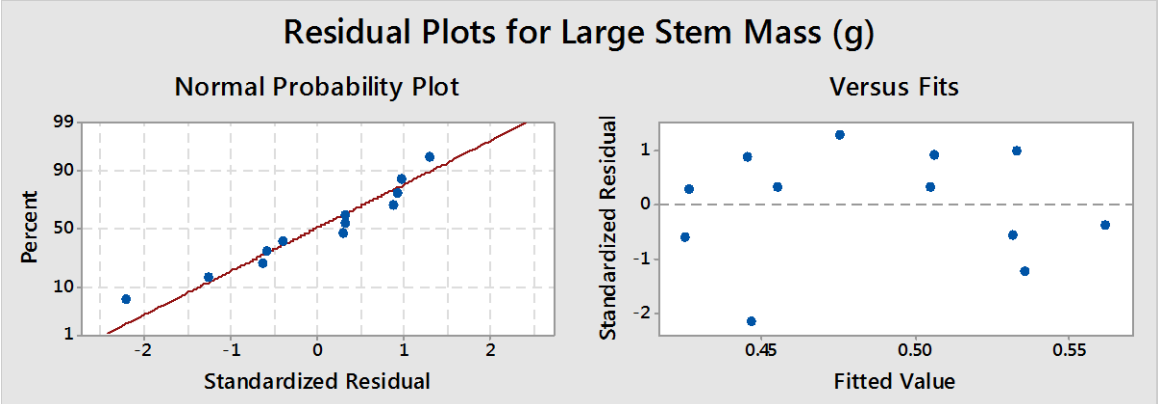
4.6.2 Drought Trial 3B barley: Small Stem Length



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	798.7	399.4	3.02	0.124
Treatment	3	418.6	139.5	1.05	0.435
Error	6	794.0	132.3		
Total	11	2011.3			

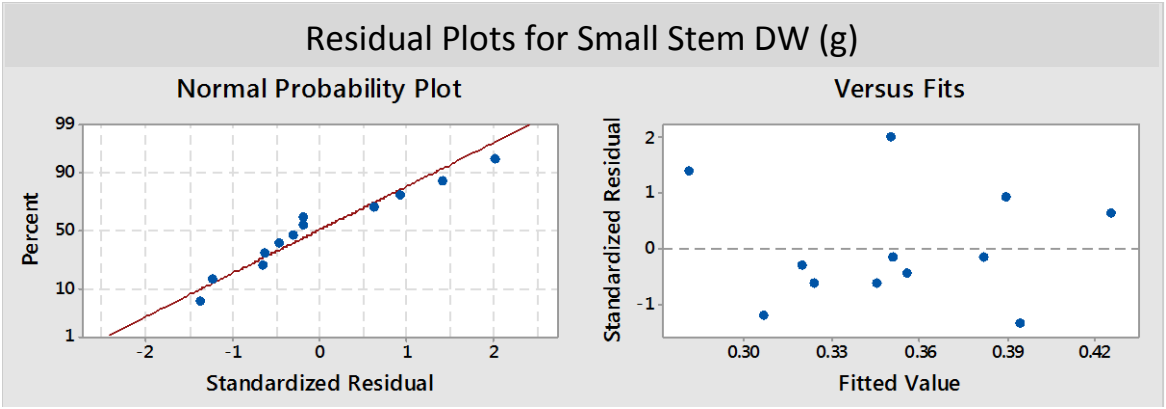
4.6.3 Drought Trial 3B barley: Large Stem DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.002404	0.001202	0.32	0.741
Treatment	3	0.022604	0.007535	1.98	0.219
Error	6	0.022884	0.003814		
Total	11	0.047892			

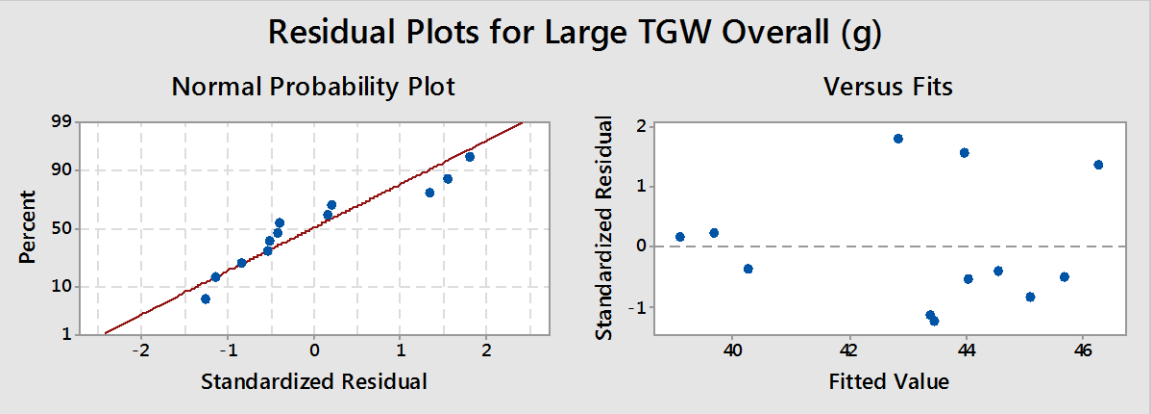
4.6.4 Drought Trial 3B barley: Small Stem DW



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.010016	0.005008	5.20	0.049
Treatment	3	0.008507	0.002836	2.94	0.121
Error	6	0.005783	0.000964		
Total	11	0.024306			

4.6.5 Drought Trial 3B barley: Large TGW Overall



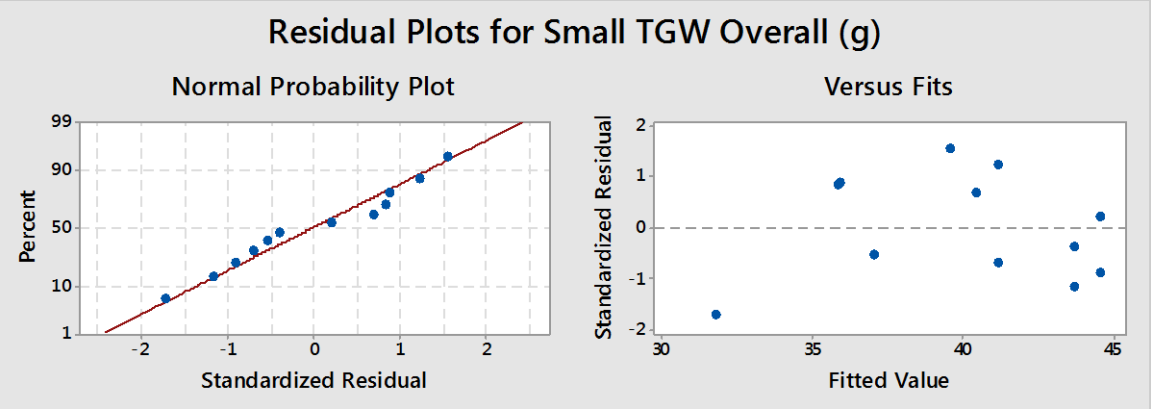
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	2.864	1.432	0.56	0.599
Treatment	3	58.310	19.437	7.59	**0.018**
Error	6	15.365	2.561		
Total	11	76.539			

Treatment / Dunnett (two sided) / Analysis of the differences between the control category Control and the other categories with a confidence interval of 95%:

Category	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs INCYDE 50 µM	-1.723	-1.319	3.099	4.050	0.467	No
Control vs CPPU 100 µM	4.316	3.303	3.099	4.050	0.039	Yes
Control vs TDZ-K 50 µM	0.532	0.407	3.099	4.050	0.954	No

4.6.6 Drought Trial 3B barley: Small TGW Overall



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	45.37	22.686	8.02	0.020
Treatment	3	137.40	45.799	16.20	**0.003**
Error	6	16.96	2.827		
Total	11	199.73			

Treatment / Dunnett (two sided) / Analysis of the differences between the control category Control and the other categories with a confidence interval of 95%:						
Category	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs TDZ-K 50 µM	-3.413	-2.486	3.099	4.255	0.109	No
Control vs INCYDE 50 µM	-2.527	-1.841	3.099	4.255	0.249	No
Control vs CPPU 100 µM	5.280	3.846	3.099	4.255	0.021	Yes

4.6.7 Drought Trial 3B barley: Large No. Grains per Head

Poisson Regression Analysis: Large No. Grains per Head versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	5	2.661	53.79%	2.661	0.5322	2.66	0.752
Block	2	1.267	25.62%	1.267	0.6337	1.27	0.531
Treatment	3	1.394	28.17%	1.394	0.4646	1.39	0.707
Error	6	2.286	46.21%	2.286	0.3810		
Total	11	4.947	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	1.888	0.280	(1.339, 2.437)	6.74	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	-0.107	0.306	(-0.707, 0.492)	-0.35	0.726	1.35
Block3	0.211	0.283	(-0.344, 0.765)	0.74	0.457	1.35
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
CPPU 100 µM	-0.055	0.315	(-0.672, 0.563)	-0.17	0.862	1.41
INCYDE 50 µM	-0.296	0.337	(-0.955, 0.364)	-0.88	0.379	1.36
TDZ-K 50 µM	-0.315	0.338	(-0.979, 0.348)	-0.93	0.351	1.36

Regression Equation

Large No. Grains per Head = exp(Y')

Y' = 1.888 +0.0Block_Block1 -0.107Block_Block2 +0.211Block_Block3
+0.0Treatment_Control -0.055Treatment_CPPU 100 µM -0.296Treatment_INCYDE 50 µM
-0.315Treatment_TDZ-K 50 µM

Goodness-o*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	6	2.28607	0.38101	2.29	0.892
Pearson	6	2.26445	0.37741	2.26	0.894

4.6.8 Drought Trial 3B barley: Small No. Grains per Head

Poisson Regression Analysis: Small No. Grains per Head versus Block, Treatment

Deviance Table

Source	DF	Seq Dev	Contribution	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	5	2.1844	48.12%	2.1844	0.4369	2.18	0.823
Block	2	1.5962	35.16%	1.5962	0.7981	1.60	0.450
Treatment	3	0.5882	12.96%	0.5882	0.1961	0.59	0.899
Error	6	2.3554	51.88%	2.3554	0.3926		
Total	11	4.5398	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	Z-Value	*P*-Value	VIF
Constant	1.458	0.367	(0.739, 2.177)	3.97	0.000	
Block						
Block1	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
Block2	-0.509	0.411	(-1.313, 0.296)	-1.24	0.215	1.20
Block3	-0.259	0.381	(-1.006, 0.488)	-0.68	0.497	1.20
Treatment						
Control	0.000000	0.000000	(0.000000, 0.000000)	*	*	*
CPPU 100 µM	-0.171	0.463	(-1.078, 0.737)	-0.37	0.712	1.42
INCYDE 50 µM	0.057	0.437	(-0.799, 0.913)	0.13	0.896	1.47
TDZ-K 50 µM	-0.256	0.474	(-1.184, 0.673)	-0.54	0.590	1.40

Regression Equation

Small No. Grains per Head = exp(Y')

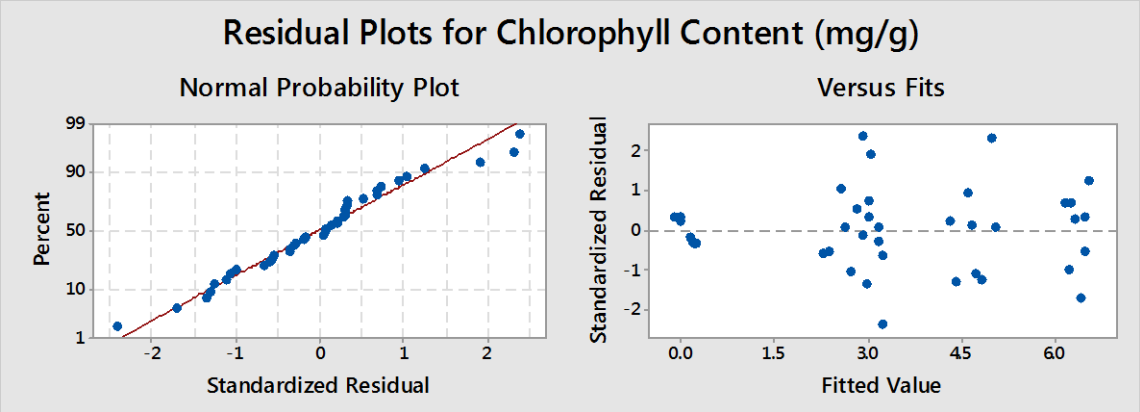
Y' = 1.458 +0.0Block_Block1 -0.509Block_Block2 -0.259Block_Block3
+0.0Treatment_Control -0.171Treatment_CPPU 100 µM +0.057Treatment_INCYDE 50 µM
-0.256Treatment_TDZ-K 50 µM

Goodness-o*F*-Fit Tests

Test	DF	Estimate	Mean	Chi-Square	*P*-Value
Deviance	6	2.35536	0.39256	2.36	0.884
Pearson	6	2.21649	0.36942	2.22	0.899

4.7 Wheat pot trial Chlorophyll

4.7.1 Wheat pot trial GS 39: Flag

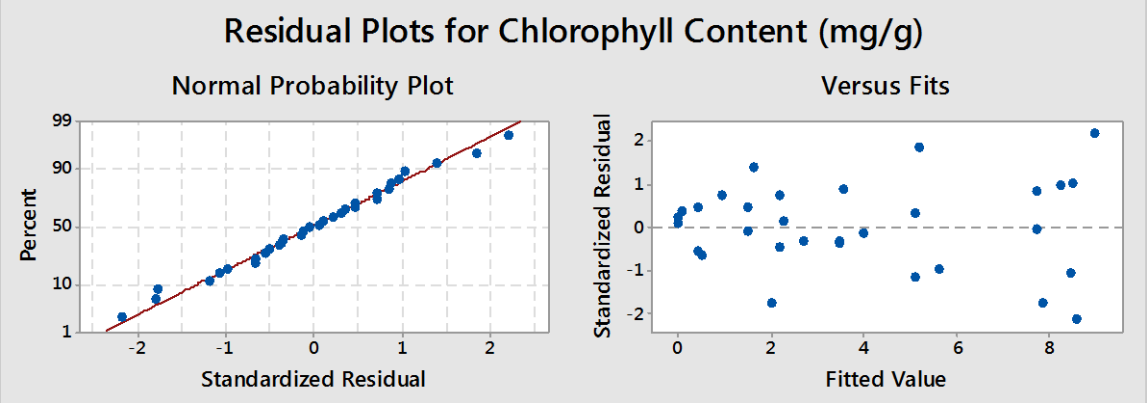


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	0.690	0.2300	0.74	0.539
Time leaf acquired	4	179.233	44.8083	143.73	0.000
Treatment	1	0.208	0.2080	0.67	0.421
Time leaf acquired*Treatment	4	0.512	0.1280	0.41	0.799
Error	27	8.417	0.3117		
Total	39	189.061			

Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):								
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
0D	6.384	0.197	5.979	6.789	A			
2W	4.716	0.197	4.311	5.122		B		
4W	3.095	0.197	2.690	3.500			C	
6W	2.687	0.197	2.282	3.092			C	
8W	0.059	0.197	-0.346	0.464				D
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
Time leaf acquired-0D*Treatment-Control	6.425	0.279	5.852	6.998	A			
Time leaf acquired-0D*Treatment-INCYDE 25 µM	6.343	0.279	5.770	6.916	A			
Time leaf acquired-2W*Treatment-INCYDE 25 µM	4.914	0.279	4.341	5.487		B		
Time leaf acquired-2W*Treatment-Control	4.519	0.279	3.946	5.092		B		
Time leaf acquired-4W*Treatment-Control	3.102	0.279	2.529	3.675			C	
Time leaf acquired-4W*Treatment-INCYDE 25 µM	3.088	0.279	2.515	3.661			C	
Time leaf acquired-6W *Treatment-INCYDE 25 µM	2.908	0.279	2.336	3.481			C	
Time leaf acquired-6W *Treatment-Control	2.465	0.279	1.892	3.038			C	
Time leaf acquired-8W*Treatment-Control	0.069	0.279	-0.503	0.642				D
Time leaf acquired-8W*Treatment-INCYDE 25 µM	0.048	0.279	-0.525	0.621				D
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
Time leaf acquired-0D*Treatment-Control	6.425	0.279	5.852	6.998	A			
Time leaf acquired-0D*Treatment-INCYDE 25 µM	6.343	0.279	5.770	6.916	A			
Time leaf acquired-2W*Treatment-INCYDE 25 µM	4.914	0.279	4.341	5.487		B		
Time leaf acquired-2W*Treatment-Control	4.519	0.279	3.946	5.092		B		
Time leaf acquired-4W*Treatment-Control	3.102	0.279	2.529	3.675			C	
Time leaf acquired-4W*Treatment-INCYDE 25 µM	3.088	0.279	2.515	3.661			C	
Time leaf acquired-6W *Treatment-INCYDE 25 µM	2.908	0.279	2.336	3.481			C	
Time leaf acquired-6W *Treatment-Control	2.465	0.279	1.892	3.038			C	
Time leaf acquired-8W*Treatment-Control	0.069	0.279	-0.503	0.642				D
Time leaf acquired-8W*Treatment-INCYDE 25 µM	0.048	0.279	-0.525	0.621				D

4.7.2 Wheat pot trial GS 39: Primary

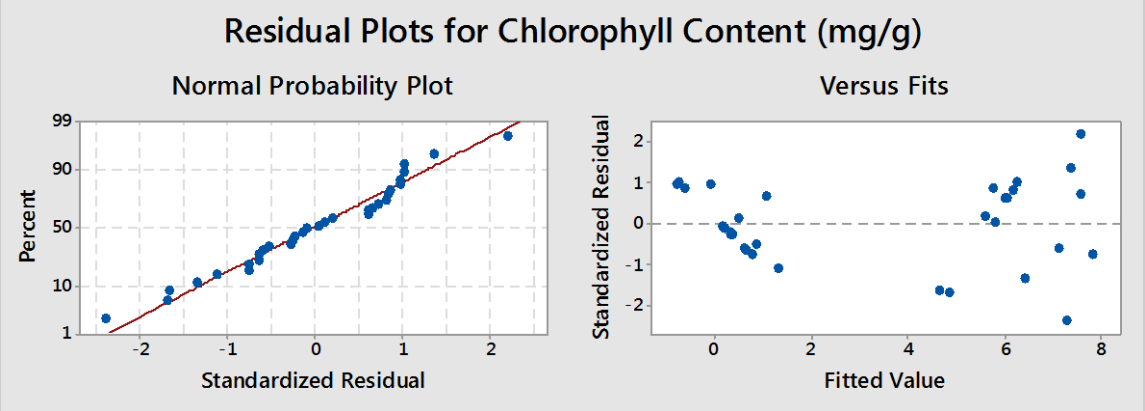


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	1.430	0.4767	0.78	0.516
Time leaf acquired	3	284.371	94.7903	155.83	0.000
Treatment	1	3.433	3.4330	5.64	0.027
Time leaf acquired*Treatment	3	4.318	1.4393	2.37	0.100
Error	21	12.774	0.6083		
Total	31	306.326			

Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):									
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups				
0D	8.275	0.276	7.702	8.849	A				
2W	4.441	0.276	3.868	5.015		B			
4W	2.002	0.276	1.428	2.575			C		
6W	0.353	0.276	-0.220	0.927				D	
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups				
Control	4.095	0.195	3.690	4.501	A				
INCYDE 25 µM	3.440	0.195	3.035	3.846		B			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups				
Time leaf acquired-0D*Treatment-Control	8.647	0.390	7.836	9.458	A				
Time leaf acquired-0D*Treatment-INCYDE 25 µM	7.903	0.390	7.092	8.714	A				
Time leaf acquired-2W*Treatment-Control	5.259	0.390	4.448	6.070		B			
Time leaf acquired-2W*Treatment-INCYDE 25 µM	3.623	0.390	2.812	4.434		B	C		
Time leaf acquired-4W*Treatment-Control	2.340	0.390	1.529	3.151			C	D	
Time leaf acquired-4W*Treatment-INCYDE 25 µM	1.664	0.390	0.853	2.475				D	E
Time leaf acquired-6W *Treatment-INCYDE 25 µM	0.571	0.390	-0.240	1.382				D	E
Time leaf acquired-6W *Treatment-Control	0.136	0.390	-0.675	0.947					E

4.7.3 Wheat pot trial GS 39: Secondary

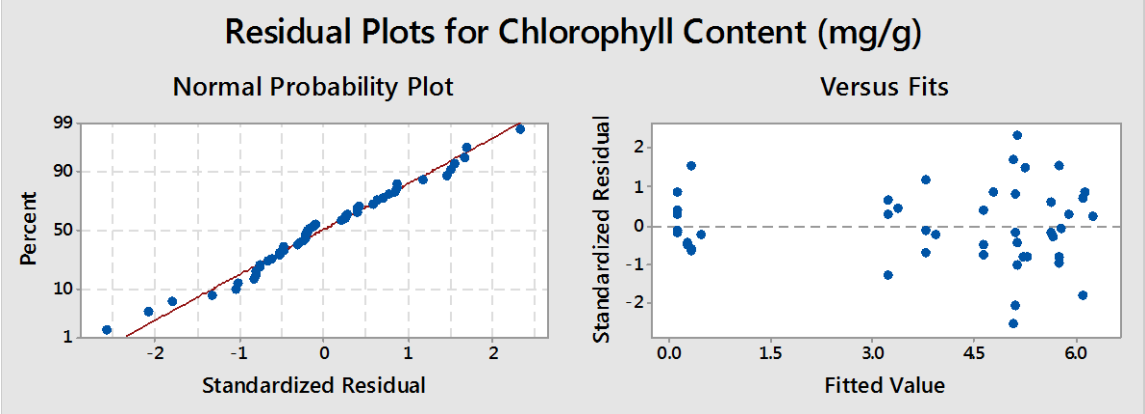


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	8.982	2.994	2.54	0.084
Time leaf acquired	3	308.875	102.958	87.23	0.000
Treatment	1	0.152	0.152	0.13	0.723
Time leaf acquired*Treatment	3	0.688	0.229	0.19	0.899
Error	21	24.787	1.180		
Total	31	343.484			

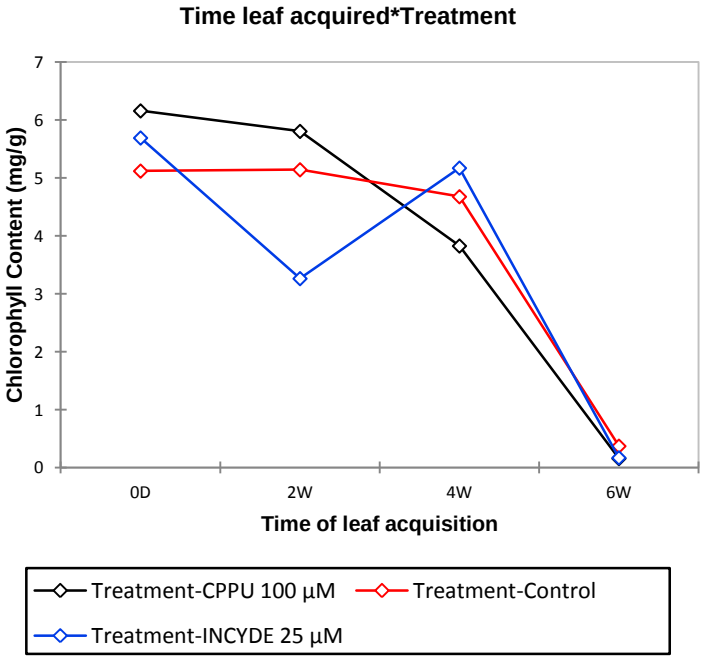
Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):							
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
0D	7.194	0.384	6.395	7.993	A		
2W	5.648	0.384	4.849	6.447		B	
4W	0.530	0.384	-0.269	1.329			C
6W	0.094	0.384	-0.705	0.893			C
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Control	3.435	0.272	2.871	4.000	A		
INCYDE 25 µM	3.297	0.272	2.733	3.862	A		
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Time leaf acquired-0D*Treatment-Control	7.329	0.543	6.200	8.459	A		
Time leaf acquired-0D*Treatment-INCYDE 25 µM	7.059	0.543	5.929	8.189	A		
Time leaf acquired-2W*Treatment-INCYDE 25 µM	5.756	0.543	4.626	6.886	A		
Time leaf acquired-2W*Treatment-Control	5.540	0.543	4.410	6.669	A		
Time leaf acquired-4W *Treatment-Control	0.803	0.543	-0.326	1.933		B	
Time leaf acquired-4W *Treatment-INCYDE 25 µM	0.257	0.543	-0.872	1.387		B	
Time leaf acquired-6W *Treatment-INCYDE 25 µM	0.118	0.543	-1.012	1.248		B	
Time leaf acquired-6W *Treatment-Control	0.070	0.543	-1.060	1.199		B	

4.7.4 Wheat pot trial GS 51: Flag



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	0.197	0.0655	0.22	0.883
Time leaf acquired	3	211.456	70.4852	234.42	0.000
Treatment	2	1.387	0.6935	2.31	0.115
Time leaf acquired*Treatment	6	18.492	3.0820	10.25	0.000
Error	33	9.922	0.3007		
Total	47	241.454			



Time leaf acquired / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll Content (mg/g)):							
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant		
0D vs 6W	5.423	24.226	2.705	**< 0.0001**	Yes		
0D vs 4W	1.098	4.905	2.705	**0.000**	Yes		
0D vs 2W	0.920	4.108	2.705	**0.001**	Yes		
2W vs 6W	4.504	20.119	2.705	**< 0.0001**	Yes		
2W vs 4W	0.179	0.797	2.705	0.855	No		
4W vs 6W	4.325	19.321	2.705	**< 0.0001**	Yes		
Tukey's d critical value:			3.825				
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
0D	5.657	0.158	5.335	5.979	A		
2W	4.737	0.158	4.415	5.059		B	
4W	4.559	0.158	4.237	4.881		B	
6W	0.234	0.158	-0.088	0.556			C

Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll Content (mg/g)):					
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
CPPU 100 µM vs INCYDE 25 µM	0.413	2.129	2.454	0.099	No
CPPU 100 µM vs Control	0.159	0.821	2.454	0.693	No
Control vs INCYDE 25 µM	0.254	1.308	2.454	0.401	No
Tukey's d critical value:			3.47		
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
CPPU 100 µM	3.987	0.137	3.709	4.266	A
Control	3.828	0.137	3.549	4.107	A
INCYDE 25 µM	3.575	0.137	3.296	3.854	A

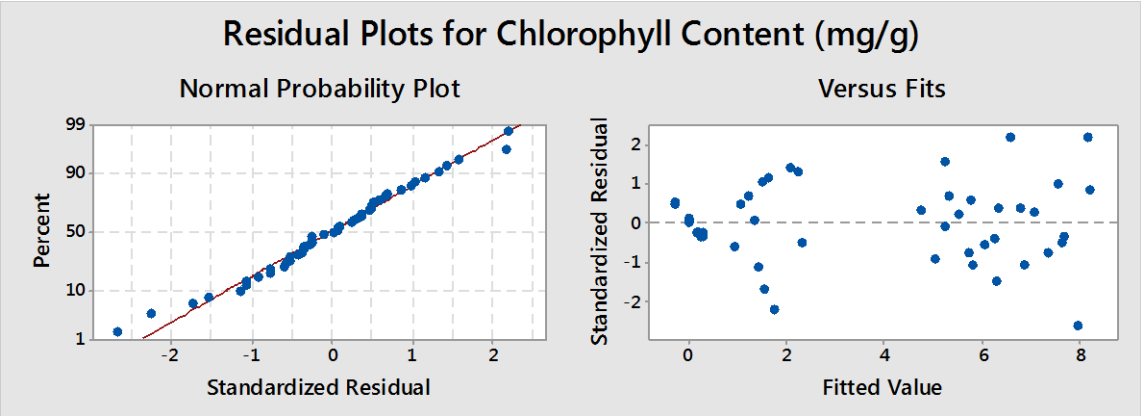
Time leaf acquired*Treatment / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll Content (mg/g)):					
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
Time leaf acquired-0D*Treatment-CPPU 100 µM vs Time leaf acquired-0D*Treatment-Control	1.039	2.679	3.511	0.280	No
Time leaf acquired-0D*Treatment-CPPU 100 µM vs Time leaf acquired-0D*Treatment-INCYDE 25 µM	0.467	1.205	3.511	0.985	No
Time leaf acquired-0D*Treatment-INCYDE 25 µM vs Time leaf acquired-0D*Treatment-Control	0.571	1.474	3.511	0.938	No
Time leaf acquired-2W*Treatment-CPPU 100 µM vs Time leaf acquired-2W*Treatment-Control	0.663	1.709	3.511	0.851	No
Time leaf acquired-2W*Treatment-Control vs Time leaf acquired-2W*Treatment-INCYDE 25 µM	1.878	4.844	3.511	**0.001**	Yes

Time leaf acquired-2W*Treatment-Control vs Time leaf acquired-4W*Treatment-CPPU 100 µM	1.315	3.392	3.511	0.066	No				
Time leaf acquired-4W*Treatment-INCYDE 25 µM vs Time leaf acquired-4W*Treatment-CPPU 100 µM	1.344	3.467	3.511	0.055	No				
Time leaf acquired-4W*Treatment-INCYDE 25 µM vs Time leaf acquired-4W*Treatment-Control	0.494	1.274	3.511	0.977	No				
Time leaf acquired-4W*Treatment-Control vs Time leaf acquired-4W*Treatment-CPPU 100 µM	0.851	2.194	3.511	0.565	No				
Time leaf acquired-6W*Treatment-Control vs Time leaf acquired-6W*Treatment-CPPU 100 µM	0.214	0.552	3.511	1.000	No				
Time leaf acquired-6W*Treatment-Control vs Time leaf acquired-6W*Treatment-INCYDE 25 µM	0.201	0.519	3.511	1.000	No				
Time leaf acquired-6W*Treatment-INCYDE 25 µM vs Time leaf acquired-6W*Treatment-CPPU 100 µM	0.013	0.033	3.511	1.000	No				
Tukey's d critical value:			4.965						

Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll Content (mg/g)):

Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups				
Time leaf acquired-0D*Treatment-CPPU 100 µM	6.159	0.274	5.601	6.717	A				
Time leaf acquired-2W*Treatment-CPPU 100 µM	5.805	0.274	5.248	6.363	A	B			
Time leaf acquired-0D*Treatment-INCYDE 25 µM	5.692	0.274	5.134	6.249	A	B			
Time leaf acquired-4W*Treatment-INCYDE 25 µM	5.172	0.274	4.614	5.729	A	B	C		
Time leaf acquired-2W*Treatment-Control	5.143	0.274	4.585	5.700	A	B	C		
Time leaf acquired-0D*Treatment-Control	5.120	0.274	4.562	5.678	A	B	C		
Time leaf acquired-4W*Treatment-Control	4.678	0.274	4.120	5.236		B	C		
Time leaf acquired-4W*Treatment-CPPU 100 µM	3.827	0.274	3.270	4.385			C	D	
Time leaf acquired-2W*Treatment-INCYDE 25 µM	3.264	0.274	2.707	3.822				D	
Time leaf acquired-6W*Treatment-Control	0.372	0.274	-0.186	0.930					E
Time leaf acquired-6W*Treatment-INCYDE 25 µM	0.171	0.274	-0.387	0.729					E
Time leaf acquired-6W*Treatment-CPPU 100 µM	0.158	0.274	-0.400	0.716					E

4.7.5 Wheat pot trial GS 51: Primary

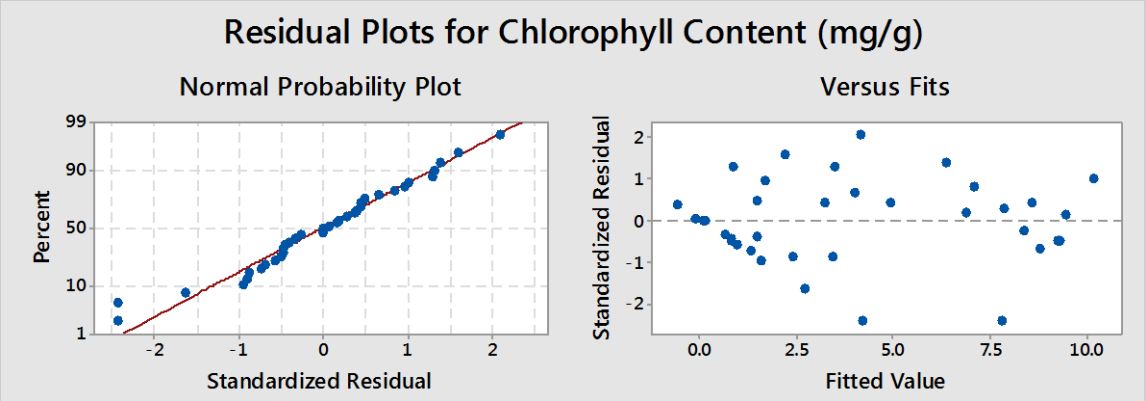


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	2.261	0.754	1.13	0.352
Time leaf acquired	3	419.196	139.732	208.98	0.000
Treatment	2	2.091	1.046	1.56	0.224
Time leaf acquired*Treatment	6	5.391	0.899	1.34	0.266
Error	33	22.065	0.669		
Total	47	451.005			

Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):									
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups				
0D	7.365	0.236	6.885	7.845	A				
2W	5.613	0.236	5.132	6.093		B			
4W	1.591	0.236	1.111	2.071			C		
6W	0.039	0.236	-0.441	0.519				D	
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups				
CPPU 100 µM	3.898	0.204	3.482	4.313	A				
Control	3.670	0.204	3.255	4.086	A				
INCYDE 25 µM	3.387	0.204	2.971	3.803	A				
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups				
Time leaf acquired-0D*Treatment-CPPU 100 µM	8.023	0.409	7.191	8.855	A				
Time leaf acquired-0D*Treatment-Control	7.430	0.409	6.598	8.261	A	B			
Time leaf acquired-0D*Treatment-INCYDE 25 µM	6.642	0.409	5.810	7.474	A	B	C		
Time leaf acquired-2W *Treatment-CPPU 100 µM	6.123	0.409	5.291	6.955	A	B	C		
Time leaf acquired-2W *Treatment-INCYDE 25 µM	5.600	0.409	4.768	6.431		B	C		
Time leaf acquired-2W *Treatment-Control	5.115	0.409	4.284	5.947			C		
Time leaf acquired-4W*Treatment-Control	2.103	0.409	1.272	2.935				D	
Time leaf acquired-4W*Treatment-CPPU 100 µM	1.401	0.409	0.569	2.232				D	E
Time leaf acquired-4W*Treatment-INCYDE 25 µM	1.269	0.409	0.437	2.100				D	E
Time leaf acquired-6W*Treatment-CPPU 100 µM	0.044	0.409	-0.788	0.876					E
Time leaf acquired-6W*Treatment-INCYDE 25 µM	0.039	0.409	-0.793	0.871					E
Time leaf acquired-6W*Treatment-Control	0.033	0.409	-0.798	0.865					E

4.7.6 Wheat pot trial GS 51: Secondary

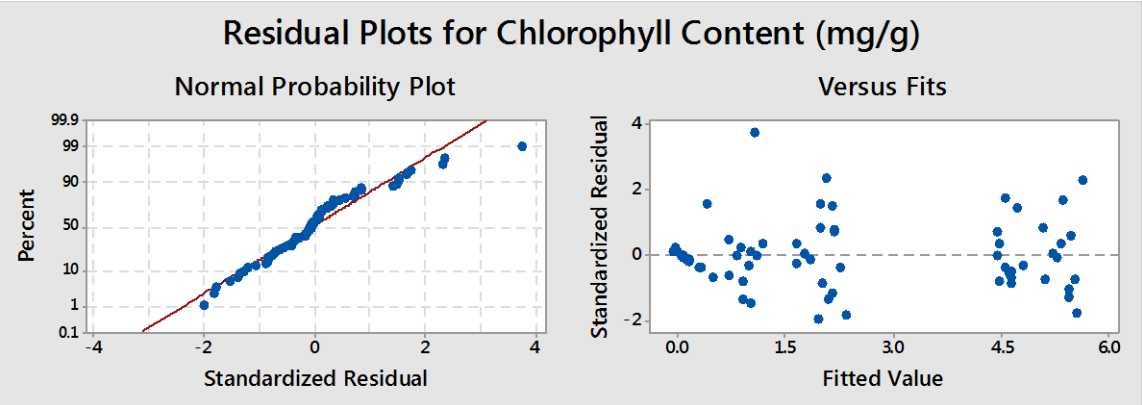


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	9.430	3.143	0.80	0.506
Time leaf acquired	2	359.613	179.806	45.77	0.000
Treatment	2	8.778	4.389	1.12	0.344
Time leaf acquired*Treatment	4	19.990	4.997	1.27	0.308
Error	24	94.280	3.928		
Total	35	492.091			

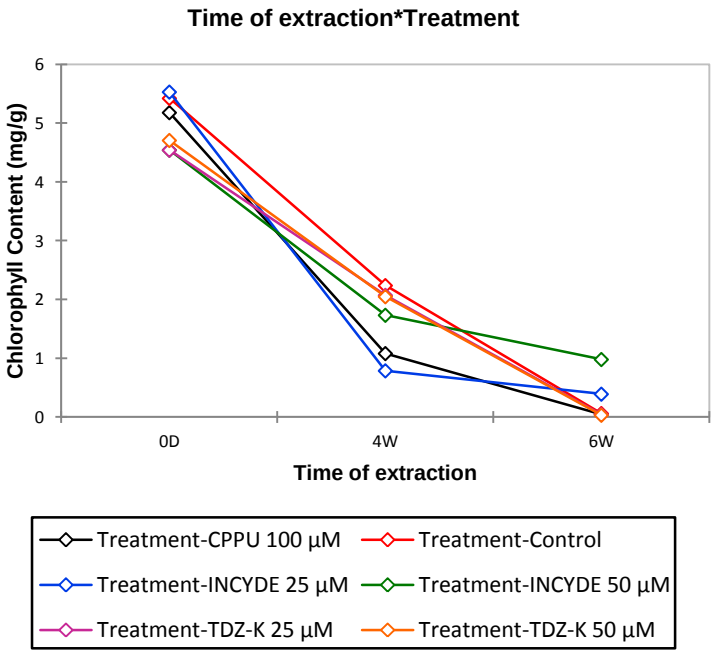
Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):								
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
0D	8.340	0.572	7.160	9.521	A			
2W	3.062	0.572	1.881	4.243		B		
4W	0.797	0.572	-0.384	1.977			C	
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
CPPU 100 µM	4.551	0.572	3.370	5.732	A			
INCYDE 25 µM	4.259	0.572	3.078	5.440	A			
Control	3.389	0.572	2.208	4.570	A			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
Time leaf acquired-0D*Treatment-CPPU 100 µM	9.435	0.991	7.389	11.480	A			
Time leaf acquired-0D*Treatment-INCYDE 25 µM	8.542	0.991	6.497	10.588	A	B		
Time leaf acquired-0D*Treatment-Control	7.044	0.991	4.999	9.090	A	B	C	
Time leaf acquired-2W*Treatment-INCYDE 25 µM	4.153	0.991	2.108	6.199		B	C	D
Time leaf acquired-2W*Treatment-CPPU 100 µM	3.387	0.991	1.342	5.432			C	D
Time leaf acquired-2W*Treatment-Control	1.646	0.991	-0.399	3.692				D
Time leaf acquired-4W*Treatment-Control	1.476	0.991	-0.570	3.521				D
Time leaf acquired-4W*Treatment-CPPU 100 µM	0.832	0.991	-1.213	2.877				D
Time leaf acquired-4W*Treatment-INCYDE 25 µM	0.082	0.991	-1.963	2.127				D

4.7.7 Wheat pot trial GS 61: Flag



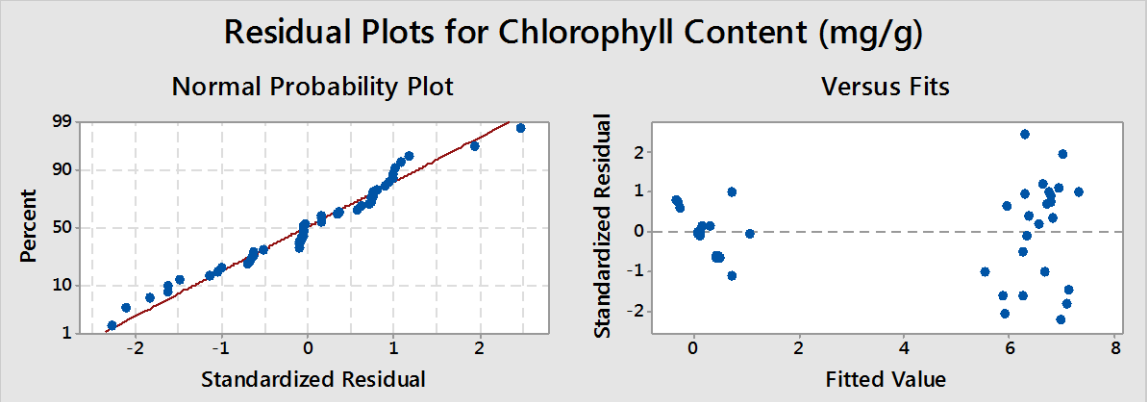
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	0.418	0.139	0.24	0.866
Time leaf acquired	2	283.357	141.679	246.35	0.000
Treatment	5	1.684	0.337	0.59	0.711
Time leaf acquired*Treatment	10	12.221	1.222	2.12	0.039
Error	51	29.331	0.575		
Total	71	327.011			



Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):							
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
0D	4.989	0.155	4.678	5.300	A		
4W	1.661	0.155	1.350	1.971		B	
6W	0.259	0.155	-0.052	0.570			C
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Control	2.575	0.219	2.135	3.014	A		
INCYDE 50 µM	2.419	0.219	1.980	2.859	A		
TDZ-K 50 µM	2.263	0.219	1.824	2.703	A		
INCYDE 25 µM	2.237	0.219	1.797	2.676	A		
TDZ-K 25 µM	2.220	0.219	1.780	2.659	A		
CPPU 100 µM	2.103	0.219	1.664	2.543	A		
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Time leaf acquired-0D*Treatment-INCYDE 25 µM	5.532	0.379	4.771	6.293	A		
Time leaf acquired-0D*Treatment-Control	5.425	0.379	4.664	6.186	A		
Time leaf acquired-0D*Treatment-CPPU 100 µM	5.182	0.379	4.421	5.943	A		
Time leaf acquired-0D*Treatment-TDZ-K 50 µM	4.709	0.379	3.947	5.470	A		
Time leaf acquired-0D*Treatment-INCYDE 50 µM	4.544	0.379	3.783	5.305	A		
Time leaf acquired-0D*Treatment-TDZ-K 25 µM	4.543	0.379	3.782	5.304	A		
Time leaf acquired-4W*Treatment-Control	2.239	0.379	1.477	3.000		B	
Time leaf acquired-4W*Treatment-TDZ-K 25 µM	2.073	0.379	1.312	2.834		B	
Time leaf acquired-4W*Treatment-TDZ-K 50 µM	2.049	0.379	1.288	2.810		B	
Time leaf acquired-4W*Treatment-INCYDE 50 µM	1.733	0.379	0.972	2.494		B	C
Time leaf acquired-4W*Treatment-CPPU 100 µM	1.082	0.379	0.321	1.843		B	C
Time leaf acquired-6W*Treatment-INCYDE 50 µM	0.980	0.379	0.219	1.742		B	C
Time leaf acquired-4W*Treatment-INCYDE 25 µM	0.788	0.379	0.026	1.549		B	C
Time leaf acquired-6W*Treatment-INCYDE 25 µM	0.391	0.379	-0.371	1.152		B	C
Time leaf acquired-6W*Treatment-Control	0.061	0.379	-0.700	0.822			C
Time leaf acquired-6W*Treatment-CPPU 100 µM	0.046	0.379	-0.715	0.807			C
Time leaf acquired-6W*Treatment-TDZ-K 25 µM	0.043	0.379	-0.718	0.804			C
Time leaf acquired-6W*Treatment-TDZ-K 50 µM	0.032	0.379	-0.729	0.793			C

4.7.8 Wheat pot trial GS 61: Primary



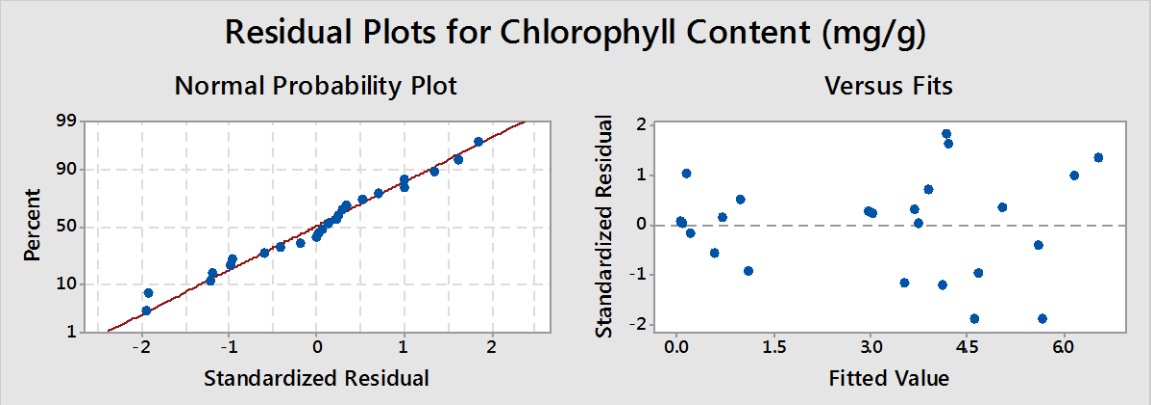
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	3.489	1.163	2.72	0.060
Time leaf acquired	1	491.194	491.194	1150.52	0.000
Treatment	5	2.631	0.526	1.23	0.316
Time leaf acquired*Treatment	5	1.645	0.329	0.77	0.578
Error	33	14.089	0.427		
Total	47	513.04			

Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):						
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
0D	6.586	0.133	6.314	6.857	A	
4W	0.188	0.133	-0.084	0.459		B
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
TDZ-K 50 µM	3.762	0.231	3.292	4.232	A	
INCYDE 50 µM	3.524	0.231	3.054	3.994	A	
CPPU 100 µM	3.427	0.231	2.957	3.897	A	
INCYDE 25 µM	3.395	0.231	2.925	3.865	A	
TDZ-K 25 µM	3.176	0.231	2.706	3.646	A	
Control	3.036	0.231	2.566	3.506	A	
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
Time leaf acquired-0D*Treatment-INCYDE 50 µM	6.974	0.327	6.310	7.639	A	
Time leaf acquired-0D*Treatment-TDZ-K 50 µM	6.817	0.327	6.153	7.482	A	
Time leaf acquired-0D*Treatment-CPPU 100 µM	6.778	0.327	6.113	7.442	A	
Time leaf acquired-0D*Treatment-INCYDE 25 µM	6.701	0.327	6.036	7.365	A	
Time leaf acquired-0D*Treatment-TDZ-K 25 µM	6.293	0.327	5.629	6.958	A	
Time leaf acquired-0D*Treatment-Control	5.951	0.327	5.286	6.616	A	
Time leaf acquired-4W*Treatment-TDZ-K 50 µM	0.707	0.327	0.043	1.372		B
Time leaf acquired-4W*Treatment-Control	0.122	0.327	-0.543	0.786		B
Time leaf acquired-4W*Treatment-INCYDE 25 µM	0.090	0.327	-0.575	0.755		B
Time leaf acquired-4W*Treatment-CPPU 100 µM	0.076	0.327	-0.589	0.740		B
Time leaf acquired-4W*Treatment-INCYDE 50 µM	0.074	0.327	-0.590	0.739		B
Time leaf acquired-4W*Treatment-TDZ-K 25 µM	0.058	0.327	-0.606	0.723		B

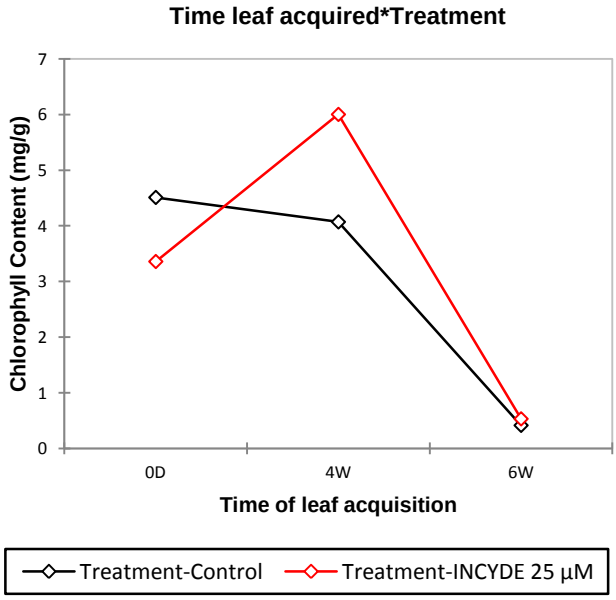
4.8 Barley pot trial Chlorophyll

4.8.1 Barley pot trial GS 51: Flag



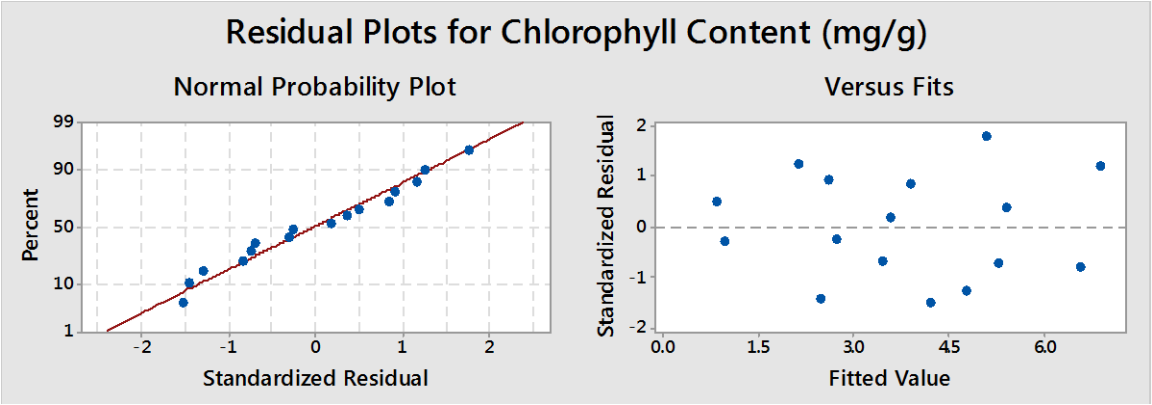
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	3.556	1.1853	1.25	0.327
Time leaf acquired	2	90.714	45.3569	47.76	0.000
Treatment	1	0.537	0.5366	0.57	0.464
Time leaf acquired*Treatment	2	9.602	4.8012	5.06	0.021
Error	15	14.245	0.9497		
Total	23	118.653			



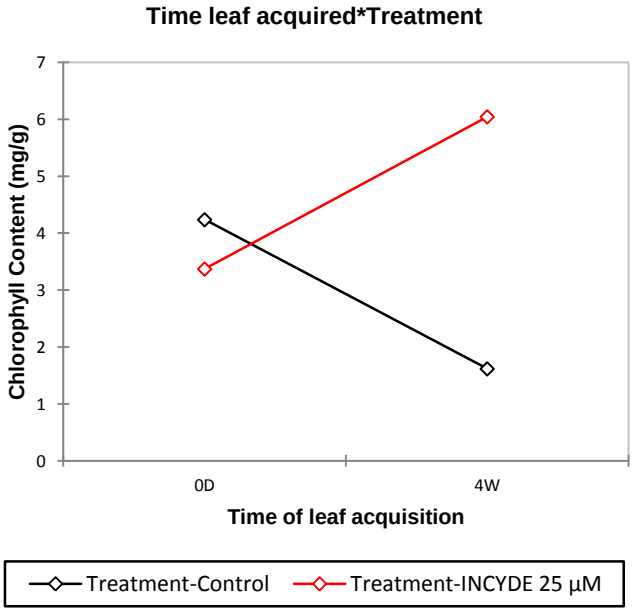
Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):							
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
4W	5.037	0.345	4.303	5.772	A		
0D	3.935	0.345	3.201	4.670	A		
6W	0.474	0.345	-0.260	1.208		B	
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
INCYDE 25 µM	3.298	0.281	2.699	3.898	A		
Control	2.999	0.281	2.400	3.599	A		
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Time leaf acquired-4W*Treatment-INCYDE 25 µM	6.003	0.487	4.964	7.041	A		
Time leaf acquired-0D*Treatment-Control	4.511	0.487	3.472	5.550	A	B	
Time leaf acquired-4W*Treatment-Control	4.072	0.487	3.033	5.110	A	B	
Time leaf acquired-0D*Treatment-INCYDE 25 µM	3.360	0.487	2.321	4.398		B	
Time leaf acquired-6W*Treatment-INCYDE 25 µM	0.533	0.487	-0.506	1.571			C
Time leaf acquired-6W*Treatment-Control	0.415	0.487	-0.623	1.454			C

4.8.2 Barley pot trial GS 51: Primary



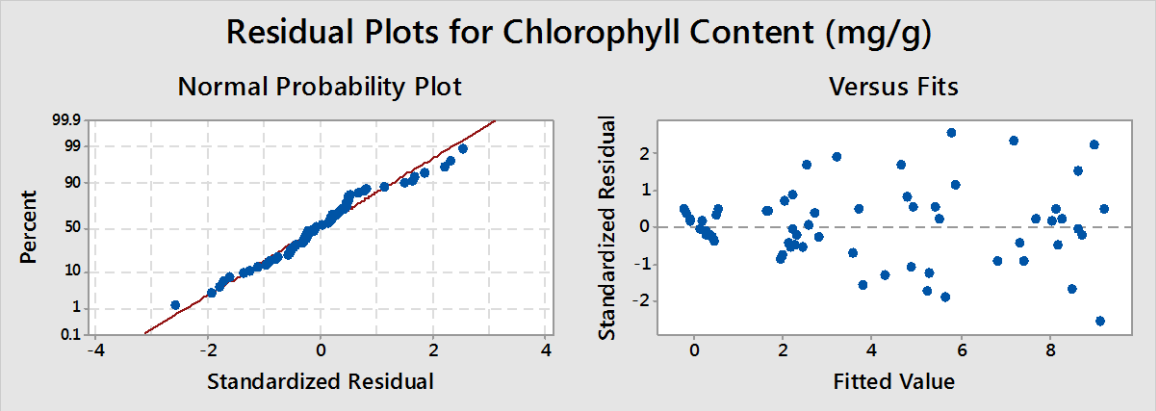
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	8.0351	2.6784	4.42	0.036
Time leaf acquired	1	0.0030	0.0030	0.00	0.946
Treatment	1	12.6713	12.6713	20.90	0.001
Time leaf acquired*Treatment	1	28.0196	28.0196	46.22	0.000
Error	9	5.4563	0.6063		
Total	15	54.1852			



Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):							
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
4W	3.830	0.275	3.207	4.453	A		
0D	3.803	0.275	3.180	4.426	A		
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
INCYDE 25 µM	4.706	0.275	4.084	5.329	A		
Control	2.927	0.275	2.304	3.549		B	
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Time leaf acquired-4W*Treatment-INCYDE 25 µM	6.043	0.389	5.163	6.924	A		
Time leaf acquired-0D*Treatment-Control	4.236	0.389	3.356	5.117		B	
Time leaf acquired-0D*Treatment-INCYDE 25 µM	3.369	0.389	2.489	4.250		B	
Time leaf acquired-4W*Treatment-Control	1.617	0.389	0.736	2.498			C

4.8.3 Barley pot trial GS 61: Flag

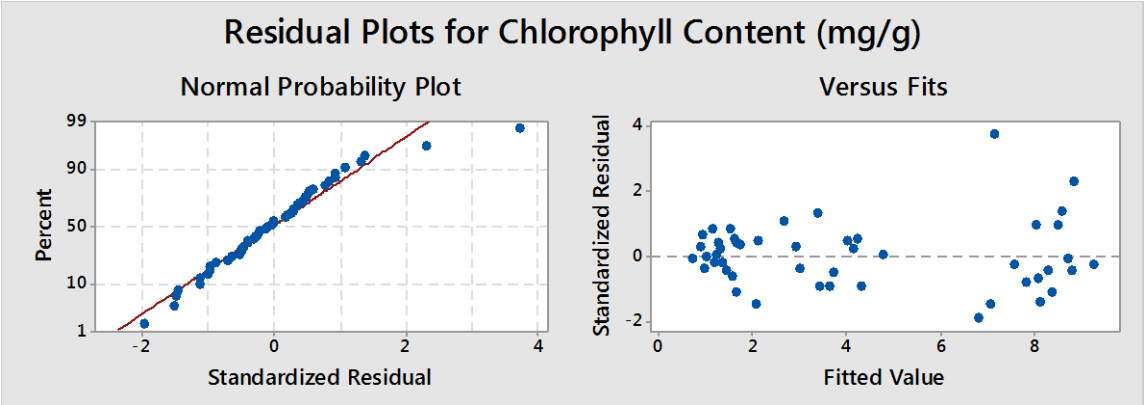


Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	3.310	1.103	1.04	0.383
Time leaf acquired	3	573.713	191.238	180.51	0.000
Treatment	3	1.506	0.502	0.47	0.702
Time leaf acquired*Treatment	9	16.637	1.849	1.74	0.106
Error	45	47.673	1.059		
Total	63	642.839			

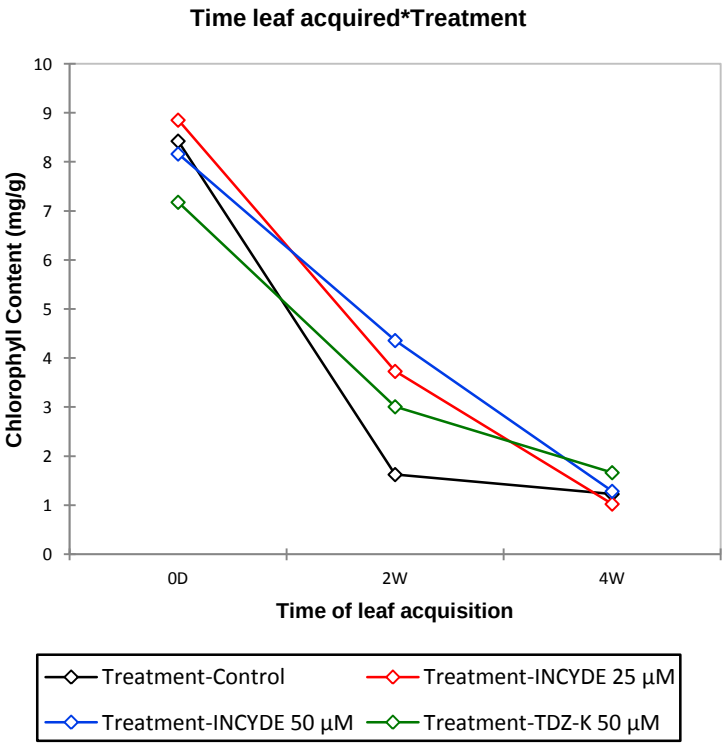
Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):											
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups						
0D	8.221	0.257	7.703	8.740	A						
2W	4.810	0.257	4.291	5.328		B					
4W	2.231	0.257	1.713	2.750			C				
6W	0.215	0.257	-0.303	0.734				D			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups						
INCYDE 25 µM	4.118	0.257	3.600	4.637	A						
Control	3.832	0.257	3.314	4.351	A						
TDZ-K 50 µM	3.828	0.257	3.309	4.346	A						
INCYDE 50 µM	3.699	0.257	3.181	4.217	A						
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups						
Time leaf acquired-0D*Treatment-INCYDE 50 µM	9.050	0.515	8.013	10.086	A						
Time leaf acquired-0D*Treatment-INCYDE 25 µM	8.542	0.515	7.505	9.578	A						
Time leaf acquired-0D*Treatment-Control	8.069	0.515	7.032	9.105	A	B					
Time leaf acquired-0D*Treatment-TDZ-K 50 µM	7.225	0.515	6.188	8.261	A	B	C				
Time leaf acquired-2W*Treatment-INCYDE 25 µM	5.652	0.515	4.616	6.689		B	C	D			
Time leaf acquired-2W*Treatment-TDZ-K 50 µM	5.324	0.515	4.288	6.361			C	D			
Time leaf acquired-2W*Treatment-Control	4.667	0.515	3.630	5.703			C	D	E		
Time leaf acquired-2W*Treatment-INCYDE 50 µM	3.595	0.515	2.558	4.631				D	E	F	
Time leaf acquired-4W*Treatment-TDZ-K 50 µM	2.592	0.515	1.555	3.628					E	F	G
Time leaf acquired-4W*Treatment-Control	2.313	0.515	1.277	3.350					E	F	G
Time leaf acquired-4W*Treatment-INCYDE 50 µM	2.035	0.515	0.999	3.072					E	F	G
Time leaf acquired-4W*Treatment-INCYDE 25 µM	1.985	0.515	0.949	3.022						F	G
Time leaf acquired-6W*Treatment-INCYDE 25 µM	0.294	0.515	-0.743	1.330							G
Time leaf acquired-6W*Treatment-Control	0.281	0.515	-0.755	1.318							G
Time leaf acquired-6W*Treatment-TDZ-K 50 µM	0.170	0.515	-0.867	1.206							G
Time leaf acquired-6W*Treatment-INCYDE 50 µM	0.117	0.515	-0.920	1.154							G

4.8.4 Barley pot trial GS 61: Primary



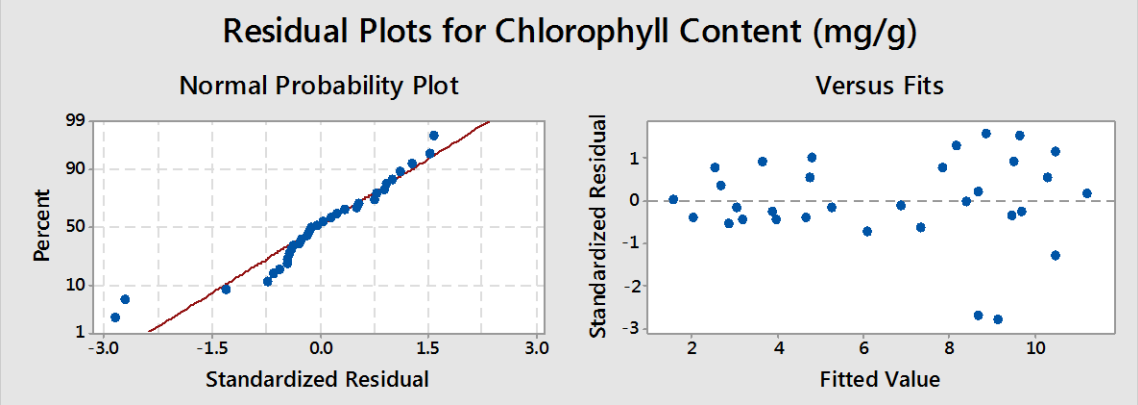
Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	3.669	1.223	1.41	0.258
Time leaf acquired	2	401.222	200.611	230.90	**0.000**
Treatment	3	6.354	2.118	2.44	0.082
Time leaf acquired*Treatment	6	17.099	2.850	3.28	**0.012**
Error	33	28.671	0.869		
Total	47	457.014			



Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):								
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
0D	8.156	0.233	7.682	8.630	A			
2W	3.183	0.233	2.708	3.657		B		
4W	1.303	0.233	0.829	1.777			C	
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
INCYDE 50 µM	4.603	0.269	4.056	5.151	A			
INCYDE 25 µM	4.538	0.269	3.990	5.085	A			
TDZ-K 50 µM	3.952	0.269	3.405	4.499	A			
Control	3.762	0.269	3.214	4.309	A			
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups			
Time leaf acquired-0D*Treatment-INCYDE 25 µM	8.854	0.466	7.906	9.802	A			
Time leaf acquired-0D*Treatment-Control	8.426	0.466	7.478	9.375	A			
Time leaf acquired-0D*Treatment-INCYDE 50 µM	8.164	0.466	7.215	9.112	A			
Time leaf acquired-0D*Treatment-TDZ-K 50 µM	7.179	0.466	6.231	8.127	A			
Time leaf acquired-2W*Treatment-INCYDE 50 µM	4.360	0.466	3.412	5.308		B		
Time leaf acquired-2W*Treatment-INCYDE 25 µM	3.732	0.466	2.784	4.680		B	C	
Time leaf acquired-2W*Treatment-TDZ-K 50 µM	3.010	0.466	2.062	3.958		B	C	D
Time leaf acquired-4W*Treatment-TDZ-K 50 µM	1.667	0.466	0.719	2.615			C	D
Time leaf acquired-2W*Treatment-Control	1.628	0.466	0.680	2.576			C	D
Time leaf acquired-4W*Treatment-INCYDE 50 µM	1.287	0.466	0.338	2.235				D
Time leaf acquired-4W*Treatment-Control	1.231	0.466	0.283	2.179				D
Time leaf acquired-4W*Treatment-INCYDE 25 µM	1.027	0.466	0.079	1.975				D

4.8.5 Barley pot trial GS 61: Secondary



Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	3	20.634	6.878	1.03	0.398
Time leaf acquired	1	233.071	233.071	35.01	0.000
Treatment	3	19.176	6.392	0.96	0.430
Time leaf acquired*Treatment	3	9.794	3.265	0.49	0.693
Error	21	139.799	6.657		
Total	31	422.474			

Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (Chlorophyll conc. (mg/g)):							
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
0D	9.263	0.645	7.922	10.604	A		
2W	3.865	0.645	2.524	5.207		B	
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
TDZ-K 50 µM	7.576	0.912	5.679	9.473	A		
INCYDE 50 µM	6.833	0.912	4.936	8.730	A		
INCYDE 25 µM	6.414	0.912	4.517	8.311	A		
Control	5.434	0.912	3.537	7.331	A		
Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Time leaf acquired-0D*Treatment-INCYDE 50 µM	10.143	1.290	7.460	12.825	A		
Time leaf acquired-0D*Treatment-TDZ-K 50 µM	9.380	1.290	6.697	12.063	A	B	
Time leaf acquired-0D*Treatment-INCYDE 25 µM	9.184	1.290	6.501	11.866	A	B	
Time leaf acquired-0D*Treatment-Control	8.345	1.290	5.663	11.028	A	B	C
Time leaf acquired-2W*Treatment-TDZ-K 50 µM	5.772	1.290	3.089	8.455	A	B	C
Time leaf acquired-2W*Treatment-INCYDE 25 µM	3.644	1.290	0.961	6.327		B	C
Time leaf acquired-2W*Treatment-INCYDE 50 µM	3.523	1.290	0.840	6.206		B	C
Time leaf acquired-2W*Treatment-Control	2.522	1.290	-0.161	5.205			C

Appendix 5: Chapter 5 statistical analyses for gene expression and LC-MS/MS analyses

5.1 Wheat cytokinin LC-MS/MS analyses

5.1.1 Wheat: *t*Z

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	14741	7370	0.36	0.720
Treatment	2	50729	25365	1.23	0.384
Error	4	82591	20648		
Total	8	148061			

5.1.2 Wheat: *t*ZR

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	134.22	67.11	1.19	0.394
Treatment	2	21.94	10.97	0.19	0.831
Error	4	226.41	56.60		
Total	8	382.57			

5.1.3 Wheat: *t*ZOG

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	24.12	12.061	1.43	0.340
Treatment	2	27.70	13.851	1.64	0.301
Error	4	33.72	8.431		
Total	8	85.55			

5.1.4 Wheat: *t*ZROG

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	1.995	0.9977	1.54	0.319
Treatment	2	1.580	0.7900	1.22	0.385
Error	4	2.587	0.6467		
Total	8	6.162			

5.1.5 Wheat: *t*ZRMP

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	562.3	281.17	5.06	0.080
Treatment	2	265.5	132.75	2.39	0.208
Error	4	222.4	55.60		
Total	8	1050.2			

5.1.6 Wheat: *t*Z9G

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	2525	1262.3	4.77	0.087
Treatment	2	2262	1130.9	4.27	0.102
Error	4	1059	264.7		
Total	8	5845			

5.1.7 Wheat: Total *t*Z types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	42248	21124	0.97	0.452
Treatment	2	41954	20977	0.97	0.454
Error	4	86693	21673		
Total	8	170895			

5.1.8 Wheat: iP

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.1201	0.06003	1.32	0.364
Treatment	2	0.2418	0.12090	2.65	0.185
Error	4	0.1825	0.04563		
Total	8	0.5444			

5.1.9 Wheat: iPR

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.3206	0.1603	1.00	0.443
Treatment	2	0.7274	0.3637	2.28	0.218
Error	4	0.6384	0.1596		
Total	8	1.6864			

5.1.10 Wheat: iPRMP

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	8.444	4.222	0.09	0.916
Treatment	2	24.005	12.003	0.26	0.786
Error	4	187.829	46.957		
Total	8	220.279			

5.1.11 Wheat: Total iP types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	14.56	7.281	0.15	0.866
Treatment	2	31.50	15.748	0.32	0.741
Error	4	194.71	48.678		
Total	8	240.77			

5.1.12 Wheat: *c*Z

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	4.169	2.085	0.64	0.573
Treatment	2	5.046	2.523	0.78	0.519
Error	4	13.000	3.250		
Total	8	22.215			

5.1.13 Wheat: *c*ZR

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	58.90	29.45	0.57	0.605
Treatment	2	130.88	65.44	1.27	0.374
Error	4	206.20	51.55		
Total	8	395.98			

5.1.14 Wheat: *c*ZOG

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	405.4	202.7	0.34	0.728
Treatment	2	492.3	246.2	0.42	0.684
Error	4	2351.9	588.0		
Total	8	3249.7			

5.1.15 Wheat: *c*ZROG

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	484.5	242.2	0.75	0.529
Treatment	2	384.5	192.2	0.60	0.594
Error	4	1292.1	323.0		
Total	8	2161.0			

5.1.16 Wheat: *c*ZRMP

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	13.091	6.546	2.81	0.173
Treatment	2	15.510	7.755	3.33	0.141
Error	4	9.320	2.330		
Total	8	37.921			

5.1.17 Wheat: Total *c*Z types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	1650.1	825.0	0.64	0.575
Treatment	2	538.5	269.2	0.21	0.821
Error	4	5183.6	1295.9		
Total	8	7372.2			

5.1.18 Wheat: DHZ

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.000422	0.000211	0.09	0.913
Treatment	2	0.002022	0.001011	0.44	0.670
Error	4	0.009111	0.002278		
Total	8	0.011556			

5.1.19 Wheat: DHZR

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.09282	0.04641	0.17	0.847
Treatment	2	0.41762	0.20881	0.78	0.519
Error	4	1.07578	0.26894		
Total	8	1.58622			

5.1.20 Wheat: DHZOG

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.2140	0.10701	1.99	0.251
Treatment	2	0.1374	0.06868	1.28	0.372
Error	4	0.2146	0.05364		
Total	8	0.5660			

5.1.21 Wheat: DHZROG

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	5.893	2.9467	1.75	0.285
Treatment	2	1.887	0.9436	0.56	0.611
Error	4	6.750	1.6876		
Total	8	14.531			

5.1.22 Wheat: DHZ7G

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	14.235	7.118	1.42	0.342
Treatment	2	4.746	2.373	0.47	0.654
Error	4	20.030	5.007		
Total	8	39.011			

5.1.23 Wheat: DHZ9G

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.000156	0.000078	0.25	0.790
Treatment	2	0.000156	0.000078	0.25	0.790
Error	4	0.001244	0.000311		
Total	8	0.001556			

5.1.24 Wheat: Total DHZ types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	19.649	9.8243	1.06	0.428
Treatment	2	1.167	0.5836	0.06	0.940
Error	4	37.217	9.3042		
Total	8	58.033			

5.1.25 Wheat: B+R *t*Z types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	17601	8800	0.39	0.701
Treatment	2	52535	26267	1.16	0.401
Error	4	90633	22658		
Total	8	160769			

5.1.26 Wheat: B+R iP types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.8325	0.4162	1.52	0.323
Treatment	2	1.2339	0.6169	2.25	0.221
Error	4	1.0957	0.2739		
Total	8	3.1620			

5.1.27 Wheat: B+R *c*Z types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	31.95	15.98	0.15	0.864
Treatment	2	145.85	72.93	0.69	0.553
Error	4	423.00	105.75		
Total	8	600.81			

5.1.28 Wheat: B+R DHZ types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.1011	0.05053	0.16	0.859
Treatment	2	0.4650	0.23250	0.73	0.538
Error	4	1.2813	0.32033		
Total	8	1.8474			

5.1.29 Wheat: Total active CKs (B+R)

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	18494	9247	0.37	0.713
Treatment	2	56827	28414	1.13	0.408
Error	4	100469	25117		
Total	8	175791			

5.1.30 Wheat: Total CK bases

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	15127	7564	0.36	0.720
Treatment	2	51166	25583	1.21	0.388
Error	4	84625	21156		
Total	8	150918			

5.1.31 Wheat: Total CK ribosides

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	170.7	85.33	0.50	0.640
Treatment	2	190.5	95.23	0.56	0.612
Error	4	683.8	170.94		
Total	8	1044.9			

5.1.32 Wheat: Total CK nucleotides

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	545.4	272.7	1.99	0.251
Treatment	2	253.1	126.6	0.92	0.468
Error	4	547.9	137.0		
Total	8	1346.5			

5.1.33 Wheat: Total CK *O*-glucosides

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	2528	1264	0.60	0.592
Treatment	2	2250	1125	0.53	0.623
Error	4	8432	2108		
Total	8	13211			

5.1.34 Wheat: Total CK *N*-glucosides

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	2909	1454.4	5.34	0.074
Treatment	2	2072	1036.1	3.81	0.119
Error	4	1089	272.3		
Total	8	6070			

5.1.35 Wheat: Total cytokinins

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	46456	23228	1.22	0.386
Treatment	2	42172	21086	1.11	0.415
Error	4	76297	19074		
Total	8	164925			

5.2 Barley cytokinin LC-MS/MS analyses

5.2.1 Barley: *t*Z

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	16307	8153	1.03	0.493
Treatment	1	20441	20441	2.58	0.249
Error	2	15837	7919		
Total	5	52585			

5.2.2 Barley: *t*ZR

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	196.9	98.44	0.01	0.990
Treatment	1	1090.0	1089.99	0.11	0.771
Error	2	19650.2	9825.09		
Total	5	20937.0			

5.2.3 Barley: *t*ZOG

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	329.16	164.58	3.90	0.204
Treatment	1	97.85	97.85	2.32	0.267
Error	2	84.39	42.19		
Total	5	511.39			

5.2.4 Barley: *t*ZROG

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	15.262	7.631	2.83	0.261
Treatment	1	6.742	6.742	2.50	0.255
Error	2	5.398	2.699		
Total	5	27.402			

5.2.5 Barley: *t*ZRMP

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	817.05	408.53	0.18	0.849
Treatment	1	0.87	0.87	0.00	0.986
Error	2	4581.29	2290.64		
Total	5	5399.21			

5.2.6 Barley: *t*Z9G

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	160.4	80.22	0.42	0.702
Treatment	1	534.7	534.68	2.82	0.235
Error	2	378.5	189.27		
Total	5	1073.7			

5.2.7 Barley: Total *t*Z types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	31359	15680	0.28	0.782
Treatment	1	5666	5666	0.10	0.781
Error	2	112482	56241		
Total	5	149507			

5.2.8 Barley: iP

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.3058	0.1529	0.69	0.592
Treatment	1	0.1734	0.1734	0.78	0.470
Error	2	0.4429	0.2215		
Total	5	0.9221			

5.2.9 Barley: iPR

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	1.5381	0.7691	1.36	0.424
Treatment	1	0.1040	0.1040	0.18	0.710
Error	2	1.1345	0.5673		
Total	5	2.7767			

5.2.10 Barley: iPRMP

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	1715.75	857.877	13.18	0.071
Treatment	1	6.85	6.848	0.11	0.776
Error	2	130.15	65.077		
Total	5	1852.76			

5.2.11 Barley: Total iP types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	1838.86	919.43	11.23	0.082
Treatment	1	11.26	11.26	0.14	0.746
Error	2	163.73	81.86		
Total	5	2013.85			

5.2.12 Barley: *c*Z

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.04003	0.02002	0.38	0.726
Treatment	1	0.21282	0.21282	4.02	0.183
Error	2	0.10583	0.05292		
Total	5	0.35868			

5.2.13 Barley: *c*ZR

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	1.740	0.8702	1.41	0.414
Treatment	1	16.138	16.1376	26.23	**0.036**
Error	2	1.231	0.6154		
Total	5	19.109			

Treatment / Dunnett (two sided) / Analysis of the differences between the control category Treatment-Control and the other categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs INCYDE 50 µM	3.280	5.121	4.303	2.756	**0.036**	Yes

5.2.14 Barley: *c*ZOG

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	1368.7	684.3	56.01	0.018
Treatment	1	11685.6	11685.6	956.39	**0.001**
Error	2	24.4	12.2		
Total	5	13078.7			

Treatment / Dunnett (two sided) / Analysis of the differences between the control category Treatment-Control and the other categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs INCYDE 50 µM	-88.263	-30.925	4.303	12.280	**0.001**	Yes

5.2.15 Barley: *c*ZROG

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	415.4	207.7	0.91	0.525
Treatment	1	307.2	307.2	1.34	0.367
Error	2	458.3	229.1		
Total	5	1180.8			

5.2.16 Barley: *c*ZRMP

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	12.1254	6.0627	0.42	0.702
Treatment	1	0.5891	0.5891	0.04	0.858
Error	2	28.5670	14.2835		
Total	5	41.2815			

5.2.17 Barley: Total *c*Z types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	2572.1	1286.1	4.83	0.171
Treatment	1	14864.3	14864.3	55.85	**0.017**
Error	2	532.3	266.1		
Total	5	17968.7			

Treatment / Dunnett (two sided) / Analysis of the differences between the control category Treatment-Control and the other categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs INCYDE 50 µM	-99.547	-7.473	4.303	57.313	**0.017**	Yes

5.2.18 Barley: DHZ

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.009433	0.004717	5.78	0.148
Treatment	1	0.035267	0.035267	43.18	**0.022**
Error	2	0.001633	0.000817		
Total	5	0.046333			

Treatment / Dunnett (two sided) / Analysis of the differences between the control category Treatment-Control and the other categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs INCYDE 50 µM	0.153	6.571	4.303	0.100	**0.022**	Yes

5.2.19 Barley: DHZR

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.2702	0.1351	0.88	0.531
Treatment	1	3.0960	3.0960	20.22	**0.046**
Error	2	0.3062	0.1531		
Total	5	3.6725			

Treatment / Dunnett (two sided) / Analysis of the differences between the control category Treatment-Control and the other categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs INCYDE 50 µM	1.437	4.497	4.303	1.375	**0.046**	Yes

5.2.20 Barley: DHZOG

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	3.0314	1.5157	6.95	0.126
Treatment	1	5.9800	5.9800	27.43	**0.035**
Error	2	0.4360	0.2180		
Total	5	9.4475			

Treatment / Dunnett (two sided) / Analysis of the differences between the control category Treatment-Control and the other categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs INCYDE 50 µM	1.997	5.237	4.303	1.640	**0.035**	Yes

5.2.21 Barley: DHZROG

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	100.878	50.439	22.02	0.043
Treatment	1	27.994	27.994	12.22	0.073
Error	2	4.581	2.290		
Total	5	133.453			

5.2.22 Barley: DHZ7G

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	2.7047	1.3523	4.48	0.183
Treatment	1	1.3136	1.3136	4.35	0.172
Error	2	0.6042	0.3021		
Total	5	4.6225			

5.2.23 Barley: DHZ9G

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.000275	0.000138	0.22	0.822
Treatment	1	0.000071	0.000071	0.11	0.770
Error	2	0.001271	0.000635		
Total	5	0.001617			

5.2.24 Barley: Total DHZ types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	152.24	76.120	8.33	0.107
Treatment	1	110.17	110.167	12.06	0.074
Error	2	18.27	9.135		
Total	5	280.68			

5.2.25 Barley: B+R *t*Z types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	18322	9161	0.26	0.794
Treatment	1	12092	12092	0.34	0.617
Error	2	70548	35274		
Total	5	100962			

5.2.26 Barley: B+R iP types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	2.6632	1.3316	0.90	0.525
Treatment	1	0.5460	0.5460	0.37	0.605
Error	2	2.9490	1.4745		
Total	5	6.1583			

5.2.27 Barley: B+R *c*Z types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	1.975	0.9873	1.40	0.417
Treatment	1	20.020	20.0203	28.40	**0.033**
Error	2	1.410	0.7050		
Total	5	23.405			

Treatment / Dunnett (two sided) / Analysis of the differences between the control category Treatment-Control and the other categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs INCYDE 50 µM	3.653	5.329	4.303	2.950	**0.033**	Yes

5.2.28 Barley: B+R DHZ types

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	0.3871	0.1935	1.12	0.471
Treatment	1	3.7604	3.7604	21.82	**0.043**
Error	2	0.3446	0.1723		
Total	5	4.4921			

Treatment / Dunnett (two sided) / Analysis of the differences between the control category Treatment-Control and the other categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs INCYDE 50 µM	1.583	4.671	4.303	1.458	**0.043**	Yes

5.2.29 Barley: Total active CKs (B+R)

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	18597	9298	0.26	0.794
Treatment	1	13373	13373	0.37	0.604
Error	2	71746	35873		
Total	5	103716			

5.2.30 Barley: Total CK bases

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	16424	8212	1.02	0.495
Treatment	1	20507	20507	2.55	0.251
Error	2	16083	8042		
Total	5	53014			

5.2.31 Barley: Total CK ribosides

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	174.7	87.34	0.01	0.991
Treatment	1	759.6	759.60	0.08	0.809
Error	2	19987.8	9993.88		
Total	5	20922.0			

5.2.32 Barley: Total CK nucleotides

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	4424.21	2212.10	0.84	0.542
Treatment	1	6.02	6.02	0.00	0.966
Error	2	5245.39	2622.69		
Total	5	9675.61			

5.2.33 Barley: Total CK *O*-glucosides

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	7770.0	3885.0	11.46	0.080
Treatment	1	17000.6	17000.6	50.15	**0.019**
Error	2	678.1	339.0		
Total	5	25448.6			

Treatment / Dunnett (two sided) / Analysis of the differences between the control category Treatment-Control and the other categories with a confidence interval of 95%:						
Contrast	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs INCYDE 50 µM	-106.460	-7.081	4.303	64.686	**0.019**	Yes

5.2.34 Barley: Total CK *N*-glucosides

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	139.4	69.68	0.39	0.719
Treatment	1	497.4	497.41	2.79	0.237
Error	2	357.1	178.53		
Total	5	993.8			

5.2.35 Barley: Total cytokinins

Normal distribution of residuals and equality of the variances was established with residual plots prior to ANOVA analysis with Minitab 17.

Analysis of Variance

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Block	2	79102	39551	0.58	0.635
Treatment	1	1561	1561	0.02	0.894
Error	2	137469	68735		
Total	5	218133			

5.3 PCR product confirmation

Primer	No. bands confirmed	Gel
TaGAP	1	
TaELF	1	
TaIPT2	2	
TaIPT3	1	
TaIPT5	1	
TaIPT7	2	
TaIPT8	1	
TaCKX1	2	
TaCKX2	1	
TaCKX3	1	
TaCKX4	2	
TaCKX8	2	
TaCKX10	1	
TaCKX11	2	
TaGLU1a	2	
TaRRA4	1	
TaZOG2	2	
TaCWINV1	1	
TaCWINV2	2	

Primer	No. bands confirmed	Gel
BrGAP	1	
BrELF	1	
BrIPT1	1	
BrIPT2	1	
BrIPT3	1	
BrCKX3	1	
BrCKX6	1	
BrCKX7	1	

5.3 Confirmed products in *Triticum aestivum* (left) and RCBr (right). The number of confirmed PCR bands is indicated, with a visual of the PCR products provided using 1% agarose gel.

5.4 Sequence data

5.4.1 *TaCWINV1* alignment with PCR primers

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                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            5         15         25         35         45         55         65         75
Primer_TaCWINV1F        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1   GGCCGGCCTC TGTGCCCGAG TGTGAGTGCA ATGGTGGTTC TTGGGGGAAG AGTTGCATGG GCATGCTCGG TGCTGCTGCT

                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            85         95        105        115        125        135        145        155
Primer_TaCWINV1F        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1   GCTGCAGCTC GCCGGGGCGT CGCATGTCGT CTACGAGACC CACCTCCTCG AGACGGAGGC GGCGGCGGCC GACGTGCCGC

                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                           165        175        185        195        205        215        225        235
Primer_TaCWINV1F        ---------- ---------- ---------- ---ACCACTT CCGGCCCATA A--------- ---------- ----------
Primer_TaCWINV1R        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1   CGTCAATTCT TGACGCCGAG CTGAGCACGG GGTACCACTT CCGGCCCATA AAGAACTGGA TCAACGATCC CAACGCGCCC

                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                           245        255        265        275        285        295        305        315
Primer_TaCWINV1F        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1   ATGTACTACA AGGGGTGGTA CCATTTCTTC TACCAGTACA ACCCCAAGGG GGCCGTGTGG GGCAACATCG TGTGGGCGCA

                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                           325        335        345        355        365        375        385        395
Primer_TaCWINV1F        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R        ---------- ---------- ---------- ---------- ---------- ---------- CAAGTCCGAC AAGTACGG--
LOCUS_AB196522_CWINV1   CTCGGTCTCG CGCGACCTCA TCAACTGGGT GGCGCTGGAG ACGGCCATCC AGCCCAGCAT CAAGTCCGAC AAGTACGGCT

                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                           405        415        425        435        445        455        465        475
Primer_TaCWINV1F        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1   GCTGGTCGGG CTCGGCCACC ATCCTGCGCG ACGGCACGCC GGTGATCATG TACACGGGCA TCGACCGCGC CGACATCAAC
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                      ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         485        495        505        515        525        535        545        555
Primer_TaCWINV1F      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1 TACGAGGTGC AGAACATCGC CTTCCCCAAG AACAAGTCGG ACCCGCTGCT CCGCGAGTGG GTCAAGCCCA GGAGCAACCC

                      ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         565        575        585        595        605        615        625        635
Primer_TaCWINV1F      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1 CATCATCGTG CCGGAGGGCG GCATCAACGC CACCCAGTTC CGGGACCCGA CCACCGCGTG GTACGCCGAC GGCCACTGGC

                      ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         645        655        665        675        685        695        705        715
Primer_TaCWINV1F      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1 GGCTGCTCAT CGGCGCCCTC TCGGGCGCGT CCCGCGGCGT GGCGTACGTG TACCGGAGCC GCGACTTCAT GCGGTGGACG

                      ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         725        735        745        755        765        775        785        795
Primer_TaCWINV1F      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1 CGGGTGAGGA AGCCGCTGCA CTCGGCGCCC ACGGGGATGT GGGAGTGCCC GGACCTGTAC CCGGTCACGG TGGACGGCCG

                      ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         805        815        825        835        845        855        865        875
Primer_TaCWINV1F      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1 GCAGAACGGG CTCGACACGT CGGTGACGTC CAGCCCGAGG GTGAAGCACG TGCTGAAGAA CAGCCTCGAC CTGCGCCGCT

                      ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         885        895        905        915        925        935        945        955
Primer_TaCWINV1F      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1 ACGACTACTA CACCGTCGGC ACCTACAACC GGAAGACCGA GCGGTACGTG CCGGACAACC CCGCCGGCGA CGAGCACCAC

                      ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         965        975        985        995        1005       1015       1025       1035
Primer_TaCWINV1F      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1 CTGCGGTACG ATTACGGCAA CTTCTACGCC TCCAAGACGT TCTACGACCC GATCAAGCGC CGCCGTATCC TGTGGGGCTG

                      ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
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                          1045       1055       1065       1075       1085       1095       1105       1115
Primer_TaCWINV1F          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1     GGCCAACGAG TCGGACACCG CCGTCGACGA CGTCGCCAAG GGATGGGCCG GAATCCAGGC GATTCCGAGG AAGGTTTGGC

                          ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|....
                             1125       1135       1145       1155       1165       1175       1185       1195
Primer_TaCWINV1F          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1     TGGACCCGAG TGGGAGGCAG CTGATGCAGT GGCCTGTGGA GGAGCTTGAG GCGCTGAGGG CGAAAAAGCC GGTGAGTCTC


                          ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|....
                             1205       1215       1225       1235       1245       1255       1265       1275
Primer_TaCWINV1F          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1     AAGGACAGGG TGGTGAAGCG GGGAGAGCAC GTCGAGGTCA CCGGGCTACG AAGCTCACAG GCTGACGTCG AGGTGAGCTT

                          ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|....
                             1285       1295       1305       1315       1325       1335       1345       1355
Primer_TaCWINV1F          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1     TGAGGTGCCG AGCTTGGAGG GAGCGGAGGC GTTGGACCCA GCGCTAGCCA ACGACGCCCA GAAGCTGTGC AGCGTGAGGG

                          ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|....
                             1365       1375       1385       1395       1405       1415       1425       1435
Primer_TaCWINV1F          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1     GTGCCGACGT GGAAGGCGGC GTGGGCCCCT TTGGTCTGTG GGTGCTCGCC TCGTCCAAGC TGGAGGAGAA GACGGCGGTC

                          ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|....
                             1445       1455       1465       1475       1485       1495       1505       1515
Primer_TaCWINV1F          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1     TTCTTCCGGG TGTTCAAGGC CGCGCGCAAC ATCAACAGCA CCAAGCCGGT GGTCCTCATG TGCTCCGACC CCACCACGTC

                          ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|....
                             1525       1535       1545       1555       1565       1575       1585       1595
Primer_TaCWINV1F          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1     ATCTTTGAAC CCGAACCTCT ACAAGCCGAC GTTCGCAGGC TTTGTTGATA CTGACATAGC GAAGGGCAAG ATATCTCTGA

                          ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|....
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                          1605       1615       1625       1635       1645       1655       1665       1675
Primer_TaCWINV1F          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1     GGAGCCTGAT TGATCGGTCC GTGGTCGAGA GCTTCGGGGC AGGAGGCAGG ACCTGCATCC TCTCCCGGGT CTATCCGACC

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                             1685       1695       1705       1715       1725       1735       1745       1755
Primer_TaCWINV1F          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1     CTCGCCCTAG GCAAGAACGC TCACCTTCAC GTTTTCAACA ACGGCAAGGT GGACATCAAG GTGTCACAGC TCACGGCGTG

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                             1765       1775       1785       1795       1805       1815       1825       1835
Primer_TaCWINV1F          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1     GGAGATGAAG AAGCCAGCGC TCATGAACGG TGCTTAGAAT TTAAGGTGGA TCTACATGCA GGCTTCCTAT ATATGTGCCT
Clustal Consensus

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                             1845       1855       1865       1875       1885       1895       1905       1915
Primer_TaCWINV1F          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1     GAATTTGGCT CATATTCTGT TAAATTGATC TCTAGTGAAA CGTGATAGTT CTACGTGGGT ACGTACGTAG CTAGGTTTGC
Clustal Consensus

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                             1925       1935       1945       1955       1965       1975       1985       1995
Primer_TaCWINV1F          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV1R          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196522_CWINV1     ACGTAGTATA AGTGCACTAC GAAGGGATCA TTTTGCCCGT AGGAATAATA AAAGTTCTAT GCATACTCTG CCGTGCACAT

                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|
                             2005       2015       2025       2035       2045       2055
Primer_TaCWINV1F          ---------- ---------- ---------- ---------- ---------- -----
Primer_TaCWINV1R          ---------- ---------- ---------- ---------- ---------- -----
LOCUS_AB196522_CWINV1     TGGTGGAAAG TATATATATT GCCGGACGAG TTTTAAAAAA AAAAAAAAAA AAAAA
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5.4.2 *TaCWINV2* alignment with PCR primers

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                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            5         15         25         35         45         55         65         75
Primer_TaCWINV2F        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2   CGGTCTCCCC CCTCCCTCGA CCCTCTTAAC TGCAAGCAAG CAATGGGGGC TCCGAAATGG GTGGTTGCGC CATTGGCGCT

                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            85         95         105        115        125        135        145        155
Primer_TaCWINV2F        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2   GCTGCTGCTC CTGCAGCTCG CCGGCGCGTC CCATGACGTC CGCCGCAGCC TCGAGGCCGA GGCGGCGTCG CCGTCCGTGC

                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            165        175        185        195        205        215        225        235
Primer_TaCWINV2F        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2   CGGCCTCCAT TCTCAGCCCC CTGCTCCGGA CCGGCTACCA CTTCCAGCCC CCCATGAACT GGATCAACGA TCCGAATGGG

                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            245        255        265        275        285        295        305        315
Primer_TaCWINV2F        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2   CCACTCTACT ACAAGGGATG GTACCACCTC TTCTACCAGT ACAACCCCAA GGGCGCCGTG TGGGGCAACA TCATCTGGGC



                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            325        335        345        355        365        375        385        395
Primer_TaCWINV2F        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2   GCACTCGGTG TCGCGCGACC TCATCAACTG GATCGCCCTC GACCCGGCCA TCAAGCCCTC CATCCCCACC GACCAGTTCG

                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            405        415        425        435        445        455        465        475
Primer_TaCWINV2F        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2   GCGTCTGGTC CGGCTCCGCC ACCATCCTCC CCAACGGCAC GGTGGCGATG CTCTACACCG GCATCGACCG CCCGGGCACC

                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            485        495        505        515        525        535        545        555
```

```
Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  AACTACCAGA TCCAGAACAT CGCCTTCCCC AAGGACCCCT CCGACCCGCT CCTCCGCGAG TGGGTCAAGC CCGGGTACAA

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          565        575        585        595        605        615        625        635
Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  CCCCATCGCC ATCCCCGAGG CCGGCATGAA CGCCACCCAG TTCCGCGACC CGACCACCGC CTGGCACGCC GGCGACGGGC

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          645        655        665        675        685        695        705        715
Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  TGTGGCGCAT GCTCGTGGGC GGCCTCAAGC CCGGCACGCT CCGCGGGATG GCCATCCTGT ACCGGAGCCG GGACTTCAAG

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          725        735        745        755        765        775        785        795
Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  CACTGGGTCC GCGCCAAGCA CCCGCTCCAC TCGGCCCTCA CCGGCATGTG GGAGTGCCCC GACTTCTTCC CCGTGCGCGA

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          805        815        825        835        845        855        865        875
Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  GCCGGGCCAC CCGGACGGCC TCGACACGTC GGAGTTTGGC CCGCACTACA AGTACGTGCT CAAGAACAGC CTCGACCTCA

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          885        895        905        915        925        935        945        955
Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  CCCGCTACGA CTACTACACG GTCGGCACCT ACAACAACCG CACGGAGCGG TACGTGCCCG ACAACCCCAC CGGCGACGTC

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          965        975        985        995        1005       1015       1025       1035
Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  TACCAGCGGC TCCAGTACGA CTACGGCAAC TTCTACGCGT CCAAGACCTT CTACGACCCC GCCAAGAACC GCCGCGTGCT

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          1045       1055       1065       1075       1085       1095       1105       1115
```

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Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  GCTCGGCTGG GCCAACGAGT CCGACAGCGT CGCCCACGAC AACGCCAAGG GATGGGCCGG CATCCACGCG ATCCCGAGGA

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          1125       1135       1145       1155       1165       1175       1185       1195
Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  AGATATGGCT GGACCCCAGC GGGAAGCAGC TGCTGCAGTG GCCCGTGGAG GAGCTGGACC AGCTGAGGGG CAAGGCTGTC

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          1205       1215       1225       1235       1245       1255       1265       1275
Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  AGCGTGGGTG ACAAGGTCGT CATGCCCGGC CAGCACTTTG AGGTCACTGG CCTACAGTCC TACCAGTCTG ACGTGGAGGT

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          1285       1295       1305       1315       1325       1335       1345       1355
Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  GAGCTTCGAG GTGCCGAGCC TGGACAAGGC GGAGCCGTTC GATCCGGCCT ACGCCAACGA CGCGCAGAAG CTCTGCGGGA

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          1365       1375       1385       1395       1405       1415       1425       1435
Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  TGAAGAACGC CGACGTCAAG GGCGGGGTGG GGCCCTTCGG CCTCTGGGTC CTGGCCTCCG ACAACCTGGC CGAGAAGACC

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          1445       1455       1465       1475       1485       1495       1505       1515
Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ----CGTCCT CATGTGCAGT GA-------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  GCCGTGTTCT TCAGAGTATT CAAGGACGGG CATGGCAAGC CTCTCGTCCT CATGTGCAGT GACCCCACCA AGTCATCTCT

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          1525       1535       1545       1555       1565       1575       1585       1595
Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  CACCGCGGGT CTATACAAGC CGACGTTTGC CGGGTTTGTC GACACCGACA TTTCGTCCGG GAAGATCTCC TTGAGAAGCT

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          1605       1615       1625       1635       1645       1655       1665       1675
```

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Primer_TaCWINV2F       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- -------CGA GAGTGTACCC ATCCA-----
LOCUS_AB196523_CWINV2  TGATCGACCG TTCGGTGGTT GAGAGCTTCG GCGCCGGAGG GAGGACCTGC ATCCTATCGA GAGTGTACCC ATCCATGGCG

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          1685       1695       1705       1715       1725       1735       1745       1755
Primer_TaCWINV2BF      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  ATCGGGAAAG ACGCGCATCT TCACGTGTTC AACAACGGGG TGACCGATAT CAAGGTGTCC AAACTAACGG CATGGGAGAT

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          1765       1775       1785       1795       1805       1815       1825       1835
Primer_TaCWINV2BF      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  GAAGAAGCCG ATGATGAACG GCGCCTAAGT GTATATGCTT TCTTGGGAGT TTTGGTTTTG GCTTAGTGTT GTTTTATGTC

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          1845       1855       1865       1875       1885       1895       1905       1915
Primer_TaCWINV2BF      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  GACCTACACC ACGTAACTGA TTGCTCGTCT GAATGAAGAC CTCTTTGAGT ATGTAGAAGT GGCTAGAGAA ATTTGTTGTA

                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          1925       1935       1945       1955       1965       1975       1985       1995
Primer_TaCWINV2BF      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Primer_TaCWINV2R       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
LOCUS_AB196523_CWINV2  CGAGCAAAAA CATGATGATT CCACGCTGTT ATTAATAAAG ATAATAAAGA CCTTAATGAT TTTCTTAAAA AAAAAAAAAA

                       ....

Primer_TaCWINV2BF      ----
Primer_TaCWINV2R       ----
LOCUS_AB196523_CWINV2  AAAA
```

5.4.3 *TaCWINV1* sequenced PCR product aligned in BLAST (NCBI)

Triticum aestivum CWINV1SM mRNA for cell wall invertase, complete cds
Sequence ID: AB196522.1Length: 2055Number of Matches: 1

Score	Expect	Identities	GAPDHs	Strand
276 bits(149)	3e-72	159/163(98%)	3/163(1%)	Plus/Plus

```
Query  14   CGCCC-TGTTCTAC-AGGGGTGGTACCATTTCTTCTACCAGT-CAACCCCAAGGGGGCCG  70
            ||||| ||| |||| |||||||||||||||||||||||||| ||||||||||||||||
Sbjct  236  CGCCCATGTACTACAAGGGGTGGTACCATTTCTTCTACCAGTACAACCCCAAGGGGGCCG  295

Query  71   TGTGGGGCAACATCGTGTGGGCGCACTCGGTCTCGCGCGACCTCATCAACTGGGTGGCGC  130
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  296  TGTGGGGCAACATCGTGTGGGCGCACTCGGTCTCGCGCGACCTCATCAACTGGGTGGCGC  355

Query  131  TGGAGACGGCCATCCAGCCCAGCATCAAGTCCGACAAGTACGG  173
            |||||||||||||||||||||||||||||||||||||||||||
Sbjct  356  TGGAGACGGCCATCCAGCCCAGCATCAAGTCCGACAAGTACGG  398
```

5.4.4 *TaCWINV2* sequenced PCR product aligned in BLAST (NCBI)

Triticum aestivum CWINV2SM mRNA for cell wall invertase, complete cds
Sequence ID: AB196523.1Length: 2004Number of Matches: 1

Score	Expect	Identities	GAPDHs	Strand
156 bits(172)	3e-36	101/111(91%)	0/111(0%)	Plus/Plus

```
Query  45    CCAACCTCTCATCCGGGAAGATCTCCCTGAGAAGCTCCCTCCACCGTTCGGTGGTTGAGA  104
             || || | || ||||||||||||||| |||||||||   || ||||||||||||||||||
Sbjct  1565  CCGACATTTCGTCCGGGAAGATCTCCTTGAGAAGCTTGATCGACCGTTCGGTGGTTGAGA  1624

Query  105   GCTTCGGCGCCGGAGGGAAGACCTGCATCCTATCGAGAGTGTACCCATCCA  155
             |||||||||||||||| |||||||||||||||||||||||||||||||||
Sbjct  1625  GCTTCGGCGCCGGAGGGAGGACCTGCATCCTATCGAGAGTGTACCCATCCA  1675
```